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Remarks:

The applicant has subsequently filed a sequence listing and declared, that it includes no new matter.

(54) Osteoclastgenic inhibitory agent comprising interleukin-18

(57) An osteoclastgenic inhibitory agent which comprises an interleukin-18 and/or its functional equivalent. The agent can be arbitrarily used as an ingredient for

cell culture and agents for regulating bone resorption and for osteoclast-related diseases, directed to treat and/or prevent hypercalcemia, osteoclastoma, osteoporosis, etc.

Description

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The present invention relates to an osteoclastgenic inhibitory agent comprising an interleukin-18 (hereinafter abbreviated as "IL-18") or its functional equivalent.

Osteoblasts' bone formation and osteoclasts' bone resorption are well balanced in healthy living bodies, and this keeps the bone tissues in normal conditions while old bone tissues are being replaced with fresh ones without altering the original bone shape. The phenomenon plays an important role in keeping living bodies' homeostasis such as the controlling of blood calcium concentration within a desired range. Once the balance is lost, especially when the bone resorption level exceeds the bone formation level, bone-related diseases and other diseases may be induced. Therefore, elucidation of the whole mechanism of bone resorption in living bodies, particularly, elucidation of osteoclasts is greatly highlighted due to scientific and clinical significance thereof.

However, the mechanism of osteoclast formation has not yet been completely elucidated even though interleukin 1 as a promoter and interleukin 4 as an inhibitor were found. This is because, similarly as various phenomena in living bodies, osteoclast formation in living bodies is controlled by the close and complicated relationship between promoters and inhibitors. Based on these, it is greatly expected to establish an effective osteoclastgenic inhibitory agent from the viewpoint of scientific and clinical aspects.

The object of the present invention is to provide a novel and effective osteoclastgenic inhibitory agent. To solve the object the present inventors energetically studied for IL-18, i.e., one of cytokines as communication transferring substances in immune systems, which induces production of interferon-y (hereinafter abbreviated as "IFN-γ"), an important biologically active substance for immunocompetent cells, and granulocyte/macrophage colony-stimulating factor (hereinafter abbreviated as "GM-CSF"), and augments cytotoxicity and induces formation of killer cells. At the finding, IL-18 was described as an interferon-γ-inducing factor as reported by Haruki OKAMURA in Japanese Patent Kokai Nos. 27.189/96 and 193,098/96, and in *Nature*, Vol. 378, No. 6.552, pp. 88-91 (1995), and then called IL-18 according to the proposal of Shimpei USHIO et al., in *The Journal of Immunology*, Vol. 156, pp. 4,274-4,279 (1996).

The present inventors found that a particular gene, capable of inhibiting osteoclast formation from osteoclastic precursor cells *in vitro*, is specifically expressed in quantities in stroma cells derived from mouse myeloma. Their further detailed analysis revealed that (i) the gene encodes IL-18 that includes SEQ ID NO: 7 as a core sequence, (ii) IL-18 and functional equivalents thereof effectively inhibit osteoclast formation, and (iii) the inhibition is mainly due to the action of GM-CSF induced and produced by IL-18.

Based on these, the present inventors solved the present object by an osteoclastgenic inhibitory agent comprising IL-18 or its functional equivalent as an effective ingredient.

- FIG. 1 shows the structure of the recombinant DNA pKGFHH2.
- FIG. 2 shows the structure of the recombinant DNA pCSHIGIF/MUT35.
- FIG. 3 shows the structure of the recombinant DNA pCSHIGIF/MUT42.
- FIG. 4 shows the structure of the recombinant DNA pBGHuGF.
- FIG. 5 shows the structure of the recombinant DNA pKGFMH2.

In these figures. KGFHH2 cDNA means a cDNA encoding the IL-18 according to the present invention: IGIF/MUT35; a DNA encoding the IL-18 according to the present invention: IGIF/MUT42; a DNA encoding the IL-18 according to the present invention: HulGiF; a chromosomal DNA encoding the IL-18 according to the present invention: KGFMH2 cDNA; a cDNA encoding the IL-18 according to the present invention: 5S; a gene for 5S ribosomal RNA; Ptac; a tac promoter: rrnBT1T2; a termination region of a ribosomal RNA operon: AmpR; an ampicillin resistent gene: pBR322ori; a replication origin of *Escherichia coli*: CMV; a cytomegalovirus promoter: IFNss; a nucleotide sequence encoding a signal peptide for subtype α2b of human interferon-α.

The present invention relates to an osteoclastgenic inhibitory agent comprising IL-18 or its functional equivalent as an effective ingredient. The wording "IL-18" as referred to in the invention includes polypeptides with the above property independently of their sources and origins. For example, the IL-18 used in the present invention includes, as internal partial amino acid sequences, the amino acid sequences of SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3, as well as SEQ ID NO: 4 and SEQ ID NO: 5, and includes the amino acid sequence of SEQ ID NO: 6 or SEQ ID NO: 7 as a whole. The wording "functional equivalent(s)" as referred to in the present invention includes (i) those wherein one or more amino acids in the amino acid sequence of IL-18 are replaced with different amino acids, (ii) those wherein one or more amino acids are added to the N- and/or C-terminal of the amino acid sequence of IL-18, (iii) those wherein one or more amino acids in the N- and/or C-terminal regions of the amino acid sequence of IL-18 are deleted, and (v) those wherein one or more amino acids in the internal regions of the amino acid sequence of IL-18 are deleted: all of these modifications should be made within the range that does not substantially lose the property of osteoclast formation by IL-18 among the inherent property of IL-18. Examples of such functional equivalents are described along with their detailed amino acid sequences in Japanese Patent Application No. 20.906/97 by the same applicant of the present applicant, i.e., polypeptides which are capable of inducing production of interferon-gamma by immunocompe-

tent cells, wherein said polypeptides contain either amino acid sequence wherein one or more cysteines are replaced with different amino acid(s) while leaving respective consensus sequences as shown in SEQ ID NOs: 1, 2 and 4 intact, or that wherein one or more amino acids are added, removed and/or replaced at one or more sites including those in the consensus sequences but excluding those of the replaced cysteine. The different amino acids to replace the cysteine(s) are not restricted to any types, as far as the resulting polypeptide, containing an amino acid sequence replaced with the different amino acid(s), exhibits an activity of inducing production of IFN-γ by immunocompetent cells in the presence or absence of an appropriate cofactor, as the wild-type polypeptides containing SEQ ID NOs: 1, 2 and 4 as consensus partial amino acid sequences, and a stability significantly higher than that of the wild-type polypeptides. The different amino acids include serine, threonine, alanine, valine, leucine, isoleucine, histidine, tyrosine, phenylalanine, tryptophan, and methionine, among which the most preferable amino acid is serine or alanine. Embodiments of the amino acid sequences, containing SEQ ID NOs: 1, 2 and 4 as consensus partial amino acid sequences, in which one or more cysteines are to be replaced with different amino acid(s) are the wild-type polypeptides containing SEQ ID NO: 6 or 7. SEQ ID NO: 6 contains cysteines at the 38th, 68th, 76th, and 127th positions from the N-terminus. SEQ ID NO: 7 contains cysteines at the 7th, 75th, and 125th positions. The polypeptides include those containing the amino acid sequence of any one of SEQ ID NOs: 20-26, which are derived from the wild-type polypeptide containing SEQ ID NO: 6, those containing the amino acid sequence of SEQ ID NO: 27 or 28, which are derived from the wild-type polypeptide containing the amino acid sequence of SEQ ID NO: 7, and those containing an amino acid sequence derived from any one of SEQ ID NOs: 20-28 by adding, removing, and/or replacing one or more amino acids to and/ or at position(s) excepting the positions where the cysteine(s) have been replaced while retaining the desired biological activities and stability. The wording "one or more amino acids" means the number of amino acids which conventional methods such as site-directed mutagenesis can usually add, remove or replace. The polypeptides containing any one of SEQ ID NOs: 20-28 possess both stability and biological activities significantly higher than those of the wild-type polypeptides.

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The functional equivalents as referred to in the present invention further include glycosylated polypeptides of IL-18 and the above polypeptides. Any of these IL-18 and functional equivalents thereof, both of which are included to and referred to as "IL-18" in the present invention, unless specified otherwise, can be used in the present invention independently of their origins; those prepared by separating from natural sources such as cell cultures and from artificially synthesized ones using recombinant DNA technology and peptide synthesis.

With economical viewpoint, methods of recombinant DNA technology are advantageously used: generally, desired IL-18 can be obtained by introducing DNAs encoding IL-18 into appropriate hosts derived from microorganisms, plants, and animals to form transformants, culturing the transformants in nutrient culture media in a conventional manner, and purifying the cultures by conventional methods used for purifying cytokines. Any DNAs can be used as the above DNAs as long as they contain a DNA encoding IL-18, and can be suitably selected depending on the purpose of the use of the present osteoclastgenic inhibitory agent or on the recombinant DNA technology used. For example, Japanese Patent Kokai Nos. 193,098/96, 231,598/96, and 27,189/96 by the same applicant of the present invention disclose in detail methods for producing IL-18 by culturing transformed microorganisms into which DNAs including a cDNA encoding mouse or human IL-18 are introduced; and Japanese Patent Application No. 185,305/96 by the same applicant of the present invention discloses in detail a method for producing IL-18 encoding human IL-18 by culturing transformed animal cells which have an introduced DNA that includes a chromosomal DNA encodes human IL-18. Japanese Patent Application No. 20,906/97 by the same applicant of the present invention discloses in detail a method for producing IL-18 by culturing transformed animal cells having an introduced DNA which includes a DNA encoding a functional equivalent of human IL-18.

The aforesaid recombinant DNA technology has an economical advantage, but depending on the hosts and DNA sequences used, the IL-18 thus obtained may have somewhat different physicochemical property from those of IL-18 produced and functions *in vivo*. Japanese Patent Application No. 67,434/96 by the same applicant of the present invention discloses in detail a preparation of IL-18 using established human cell lines as natural sources, and Japanese Patent Application No. 213,267/96 by the same applicant also discloses in detail the preparation using an interleukin-1β-converting enzyme. The IL-18 obtained by those preparations can be estimated to have substantially the same or equal physicochemical property to that of IL-18 that is produced and functions in *vivo*, and the yield can be estimated to be slightly lower. However, such IL-18 has an advantage that it has a fewer side effects when used as pharmaceuticals directed to administering to warm-blooded animals in general and including humans. When applying purification methods using monoclonal antibodies specific to IL-18, as disclosed in Japanese Patent Application No. 231,598/96 by the same applicant of the present invention, a relatively-high purity IL-18 can be obtained in a minimum labor and cost.

The present osteoclastgenic inhibitory agent comprising the aforesaid IL-18 includes any types and forms usable to inhibit osteoclast formation both in *vivo* and in *vitro*. The present agent can be advantageously used as ingredients for cell culture media for animal cells, which satisfactorily inhibit osteoclast formation, maintain, proliferate, and/or differentiate the desired cells: components of screening kits for bone-related therapeutic agents; bone-resorption regulatory agents and agents for osteoclast-related diseases. The bone-resorption regulatory agents include medica-

ments and health foods that exert an osteoclastgenic inhibitory activity in *vivo*, control bone resorption to normal conditions, and improve unfavorable physical conditions such as a relatively-insignificant arthralgia. The agents for osteoclast-related diseases include medicaments used to prevent and/or treat diseases caused by an excessive osteoclast formation and/or its function. Examples of such diseases are hypercalcemia, osteoclastoma, Behcet's syndrome, osteosarcoma, arthropathy, chronic rheumatoid arthritis, deformity ostitis, primary hyperthyroidism, osteopenia, and osteoporosis. Varying depending on the types of agents and diseases to be treated, the present agent is usually formulated into a liquid, paste, or solid form which contains 0.000002-100 w/w %, preferably, 0.0002-0.5 w/w % of IL-18.

The present osteoclastgenic inhibitory agent can be IL-18 alone or compositions comprising IL-18 and one or more other ingredients such as carriers, excipients, diluents, adjuvants, antibiotics, and proteins such as serum albumin and gelatin as stabilizers; saccharides such as glucose, maltose, maltotriose, maltotetraose, trehalose, sucrose, isomaltose, lactose, panose, erlose, palatinose, lactosucrose, raffinose, fructooligosaccharide, galactooligosaccharide, lentinan, dextrin, pullulan, and sugar alcohols including sorbitol, maltitol, lactitol, and maltotriitol; buffers comprising phosphates or citrates mainly: and reductants such as 2-mercaptoethanol, dithiothreitol, and reduced glutathione; and optionally biologically active substances such as interferon-a, interferon-p, interferon-γ, interleukin-2, interleukin-3, interleukin-6, interleukin-12, TNF-α, TNF-β, GM-CSF, estrogen, progesterone, chlormadinone acetate, calcitonin, somatokine, somatomedin, insulin-like growth factor, ipriflavone, parathyroid hormone (PTH), norethisterone, busulfan, ancitabine, cytarabine, fluorouracil, tetrahydrofurfuryl fluorouracil, methotrexate, vitamin D₂, active vitamin D, Krestin® or polysaccharide K, L-asparaginase, and OK-432 or Picibanil®; and calcium salts such as calcium lactate, calcium chloride, calcium monohydrogenphosphate, and L-calcium L-aspartate. When used as agents for administering to warmblooded animals in general and including humans, i.e., agents for osteoclast-related diseases, the present agent can be preferably formulated into compositions by appropriately combining with one or more of the above physiologically-acceptable substances.

The present osteoclastgenic inhibitory agent includes medicaments in a unit dose form used for administering to warm-blooded animals in general and including humans. The wording "unit dose form" means those which contain IL-18 in an amount suitable for a daily dose or in an amount up to four fold by integers or up to 1/40 fold of the dose, and those in a physically separated and formulated form suitable for prescribed administrations. Examples of such formulations are injections, liquids, powders, granules, tablets, capsules, troches, collyriums, nebulas, and suppositories.

The present agent as an osteoclastgenic inhibitory agent effectively treat and prevent osteoclast-related diseases independently of oral and parenteral administrations. Varying depending on the types and symptoms of patients' diseases, the present agent can be administered to the patients orally, intradermally, subcutaneously, muscularly, or intravenously at a dose of about $0.5 \, \mu g$ to $100 \, mg$ per shot, preferably, at a dose of about $2 \, \mu g$ to $10 \, mg$ per shot of IL-18, 2-6 fold a day or 2-10 fold a week for one day to one year.

In the below, with reference to experiments, the preparation, physicochemical property, and biological activity of the IL-18 according to the present invention are described:

Experiment 1

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Preparation of human IL-18

According to the method in Japanese Patent Kokai No. 231,598/96 by the same applicant of the present invention, an autonomously-replicable recombinant DNA, pKGFHH2, linked to a cDNA encoding human IL-18, was prepared. Dideoxyribonucleotide sequencing analyzed that, as shown in FIG. 1, in the recombinant DNA, KGFHH2 cDNA containing the base sequence of SEQ ID NO: 8 was linked to the downstream of Ptac, a Tac promoter. The recombinant DNA pKGFHH2 contained the amino acid sequences of SEQ ID NOs: 1 to 5; these amino acid sequences were respectively encoded by nucleotides 46-63, 88-105, 400-420, 151-165, and 214-228 in SEQ ID NO: 8.

According to the method in Japanese Patent Kokai No. 231,598/96, the recombinant DNA pKGFHH2 was introduced into an *Escherichia coli* Y1090 strain, ATCC 37197, and the strain was cultured. The produced polypeptide was purified by immunoaffinity chromatography to obtain a purified human IL-18 with a purity of at least 95% in a yield of about 25 mg/ε culture. According to the method in Japanese Patent Kokai No. 193,098/96 by the same applicant of the present invention, the purified human IL-18 was analyzed for biological activity and physicochemical property as indicated below: When culturing human lymphocytes, collected by a conventional manner from a healthy donor, in the presence of the purified human IL-18. IFN-γ production was observed depending on the concentration of IL-18, resulting in a confirmation that IL-18 has an activity of inducing IFN-γ production by lymphocytes as an immunocompetent cell. In accordance with the method as reported by U. K. Laemmli in *Nature*, Vol. 227, pp. 680-685 (1970), the purified IL-18 was subjected to SDS-PAGE, resulting in a major band with an IFN-γ inducing activity at a position corresponding to 18,500±3.000 daltons. The IL-18 gave a pl of 4.9±1.0 as determined by conventional chromatofocusing. Conventional analysis using "PROTEIN SEQUENCER MODEL 473A", an apparatus of Applied Biosystems, Inc., Foster City.

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USA, revealed that the IL-18 had the amino acid sequence of SEQ ID NO: 9, i.e., the amino acid sequence of SEQ ID NO: 8 where a methionine residue was linked to the N-terminus.

Experiment 2

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Preparation of human IL-18

According to the method in Japanese Patent Application No. 67.434/96 by the same applicant of the present invention, THP-1 cells, ATCC TIB 202, a human monocyte cell line derived from a male with acute monocytic leukemia, were inoculated to the dorsum subcutaneous tissues of new born hamsters, followed by feeding the hamsters for three weeks. Tumor masses, about 15 g weight each, formed in the subcutaneous tissues of each hamster, were extracted, dispersed in media, and disrupted. The polypeptide obtained from the disrupted cells was purified by immunoaffinity chromatography to obtain a purified human IL-18 in a yield of an about 50 ng/head.

Similarly, according to the method in Japanese Patent Application No. 67,434/96, the purified human IL-18 was analyzed and determined for biological activity and physicochemical property as indicated below: It was confirmed that culturing human lymphocytes, collected from healthy donors in a conventional manner, in the presence of different concentrations of the human IL-18, resulted in an IL-18 dose-dependent IFN-y production. This revealed that the human IL-18 has a biological activity of inducing IFN-γ production by lymphocytes as an immunocompetent cell. In accordance with the method as reported by U. K. Laemmli in Nature, Vol. 227, pp. 680-685 (1970), the purified human IL-18 was subjected to SDS-PAGE using 2 w/v % dithiothreitol as a reductant, resulting in a major band with an IFN-γ production inducing activity at a position corresponding to 18,000-19,500 daltons. According to the peptide map disclosed in Japanese Patent Application No. 67,434/96, the human IL-18 was treated with clostripain commercialized by Sigma Chemical Company, Missouri, USA, to obtain polypeptide fragments, followed by subjecting the fragments for fractionation to high-performance liquid chromatography (HPLC) using "ODS-120T", a column commercialized by Tosoh Corporation, Tokyo, Japan, and analyzing the amino acid sequences of the fragments from the N-terminus to reveal the following amino acid sequences of SEQ ID NOs: 10 to 13. These amino acid sequences were completely coincided with amino acids 148-157, 1-13, 45-58, and 80-96 in SEQ ID NO: 6. The data shows that the human IL-18 obtained in Experiment 2 has the amino acid sequence of SEQ ID NO: 6 and all the partial amino acid sequences of SEQ ID NOs: 1 to 5.

Experiment 3

Preparation of functional equivalents

According to the method in Japanese Patent Application No. 20.906/97 by the same applicant of the present invention, it was prepared an autonomously-replicable recombinant DNA. pCSHIGIF/MUT35, was linked to a DNA encoding a functional equivalent of human IL-18 where cysteines 38, 68, and 76 in SEQ ID NO: 6 were respectively replaced with serine, serine, and alanine. Dideoxyribonucleotide sequence analysis revealed that as shown in FIG. 2, in the recombinant DNA. DNA IGIF/MUT35 with SEQ ID NO: 14 linked to the downstream of a base sequence encoding a signal peptide of subtype α2b in human interferon-a in the same reading-frame, as reported by K. Henco et al., in *Journal of Molecular Biology,* Vol. 185, pp. 227-260 (1985), and had a stop codon for protein synthesis at further downstream. As shown in parallel in SEQ ID NO: 14, the amino acid sequence encoded by the recombinant DNA corresponded to SEQ ID NO: 6 where cysteines 38, 68, and 76 in SEQ ID NO: 6 were respectively replaced with serine, serine, and alanine. The recombinant DNA contained a nucleotide which encodes all the amino acid sequences of SEQ ID NOs: 1 to 4 and the one of SEQ ID NO: 5 where cysteine at amino acid 5 in SEQ ID NO: 5 was replaced with alanine. These amino acid sequences were respectively encoded by nucleotides 46-63. 88-105, 400-420, 151-165, and 214-228 in SEQ ID NO: 14.

According to the method in Japanese Patent Application No. 20,906/97 by the same applicant of the present invention, the recombinant DNA pCSHIGIF/MUT35 was introduced into COS-1 cells, ATCC CRL 1650, an established cell line derived from SV40 transformed African Green monkey kidney, followed by culturing the transformed cells. The produced polypeptide in the culture was purified by immunoaffinity chromatography to obtain a purified functional equivalent of human IL-18 in a yield of about 40 ng/ml culture. According to the method in Japanese Patent Application No. 20,906/97, the purified functional equivalent was analyzed and determined for biological activity and physicochemical property as indicated below: When culturing KG-1 cells, ATCC CCL 246, an established cell line derived from human acute myelogenous leukemia, in the presence of different concentrations of the purified functional equivalent of human IL-18, IFN-γ production was observed depending on the concentration of the IL-18, revealing that the IL-18 has a biological activity of inducing IFN-γ production by KG-1 cells as an immunocompetent cell. In accordance with the method as reported by U. K. Laemmli in *Nature*, Vol. 227, pp. 680-685 (1970), the purified functional equivalent was

subjected to SDS-PAGE in the presence of 2 w/v % dithiothreitol as a reductant, resulting in a major band with an IFN-yproduction inducing activity at a position corresponding to 18,000-19,500 daltons. Conventional analysis using "PRO-TEIN SEQUENCER MODEL 473A", an apparatus of Applied Biosystems. Inc., Foster City, USA, revealed that the N-terminal region of the functional equivalent had the amino acid sequence of SEQ ID NO: 15 which corresponded to the amino acid sequence in the N-terminal region as shown in parallel in SEQ ID NO: 14.

Experiment 4

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Preparation of functional equivalent

According to the method in Japanese Patent Application No. 20,906/97 by the same applicant of the present invention, it was prepared an autonomously-replicable recombinant DNA, pCSHIGIF/MUT42, which was linked to a DNA encoding for a functional equivalent of human IL-18 where cysteines 38, 68, 76, and 127 in SEQ ID NO: 6 were respectively replaced with serine, serine, alanine, and serine. Dideoxyribonucleotide sequencing revealed that, as shown in FIG. 3, in the recombinant DNA, DNA IGIF/MUT42 with SEQ ID NO: 16 linked to the downstream of a base sequence encoding a signal peptide for subtype α2b of human interferon-a in the same reading frame, as reported by K. Henco et al., in *Journal of Molecular Biology*, Vol. 185, pp. 227-260 (1985), and had a stop codon for protein synthesis at further downstream. As shown in parallel in SEQ ID NO: 16, the amino acid sequence encoded by the recombinant DNA corresponded to SEQ ID NO: 6 where cysteines 38, 68, 76, and 127 in SEQ ID NO: 6 were respectively replaced with serine, serine, alanine, and serine. The recombinant DNA contained a nucleotide sequence which encodes all the amino acid sequences of SEQ ID NOs: 1 to 4 and the one of SEQ ID NO: 5 where cysteine 5 in SEQ ID NO: 5 was replaced with alanine. These amino acid sequences were respectively encoded by nucleotides 46-63, 88-105, 400-420, 151-165, and 214-228 in SEQ ID NO: 16.

According to the method in Japanese Patent Application No. 20,906/97 by the same applicant of the present invention, the recombinant DNA pCSHIGIF/MUT42 was introduced into COS-1 cells, followed by culturing the cells. The produced polypeptide in the culture was purified by immunoaffinity chromatography to obtain a purified functional equivalent of human IL-18 in a yield of about 20 ng/ml culture. According to the method in Japanese Patent Application No. 20,906/97, the purified functional equivalent was analyzed and determined for biological activity and physicochemical property as indicated below: When cultured KG-1 cells in the presence of different concentrations of the purified functional equivalent, a dose-dependent IFN-γ production was observed, and this revealed that the functional equivalent has a biological activity of inducing IFN-γ production by KG-1 cells as an immunocompetent cell. In accordance with the method as reported by U. K. Laemmli in *Nature*, Vol. 227, pp. 680-685 (1970), the purified functional equivalent was subjected to SDS-PAGE in the presence of 2 w/v % dithiothreitol as a reductant, resulting in a major band with an IFN-γ inducing activity at a position corresponding to 18,000-19,500 daltons. Conventional analysis using "PROTEIN SEQUENCER MODEL 473A", an apparatus of Applied Biosystems, Inc., Foster City, USA, revealed that the N-terminal region of the functional equivalent had the amino acid sequence of SEQ ID NO: 15 which completely corresponded to the amino acid sequence in the N-terminal region as shown in parallel in SEQ ID NO: 16.

Experiment 5

Preparation of human IL-18

According to the method in Japanese Patent Application No. 185,305/96 by the same applicant of the present invention, an autonomously-replicable recombinant DNA, **pBGHuGF**. Iinked to a chromosomal DNA encoding human IL-18, was obtained. Dideoxyribonucleotide sequencing analysis revealed that as shown in FIG. 4, in the recombinant DNA, a chromosomal DNA, which encodes human IL-18. i.e., DNA HulGIF with SEQ ID NO: 17, was linked to the downstream of a restriction site by a restriction enzyme, *Hind* III. As shown in SEQ ID NO: 17, the chromosomal DNA HulGIF consists of 11,464 bp where the exon was fragmented by four introns positioning at nucleotides 83-1,453, 1,466-4.848, 4,984-6,317, and 6,452-11,224. Among the resting nucleotide sequence excluding these introns, nucleotides 3-11,443 from the 5'-terminus are the part that encodes a precursor of human IL-18, and nucleotides 4,866-4,983 are the part that encodes an active human IL-18. The chromosomal DNA contained nucleotides sequences encoding SEQ ID NOs: 1 to 5: these amino acid sequences were respectively encoded by nucleotides 4,911-4,928, 4,953-4,970, 11,372-11,392, 6,350-6,364, and 6,413-6.427 in SEQ ID NO: 17.

According to the method in Japanese Patent Application No. 185,305/96, the recombinant DNA pBGHuGF was introduced into CHO-K1 cells, ATCC CCL 61, an established cell line derived from Chinese hamster ovary, followed by culturing the cells. The culture supernatant was contacted with a supernatant of cell disruptant prepared from a THP-1 cell culture to produce a polypeptide which was then purified by immunoaffinity chromatography to obtain a purified human IL-18 in a yield of about 15 mg/f culture. According to the method in Japanese Patent Application No.

185,305/96, the polypeptide was analyzed and determined for biological activity and physicochemical property as indicated below: It was confirmed that human lymphocytes, which were collected from a healthy donor, produced IFN-γ depending on the purified human IL-18 concentration when cultured at different concentrations of the human IL-18, revealing that the human IL-18 has a biological activity of inducing IFN-γ production by lymphocytes as an immunocompetent cell. In accordance with the method as reported by U. K. Laemmli in *Nature*, Vol. 227, pp. 680-685 (1970), the purified human IL-18 was subjected to SDS-PAGE in the presence of 2 w/v % dithiothreitol as a reductant, resulting in a major band with an IFN-γ inducing activity at a position corresponding to 18,000-19.500 daltons. The N-terminal region of the human IL-18 contained the amino acid sequence of SEQ ID NO: 15 which completely corresponded to the amino acid sequence in the N-terminal region of SEQ ID NO: 17 for an active IL-18. Experiment 6

Preparation of mouse IL-18

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To a 0.5-ml reaction tube were added 8 μl of 25 mM magnesium chloride, 10 μl of 10 x PCR buffer, one μl of 25 mM dNTP mix, one μl of 2.5 units/μl of amplitaq DNA polymerase, one ng of a recombinant DNA, which encodes mouse IL-18 having the nucleotide sequence of SEQ ID NO: 18 and the amino acid sequence of SEQ ID NO: 7, prepared from a phage DNA clone according to the method in Japanese Patent Kokai No. 27,189/96, and adequate amounts of a sense and antisense primers having nucleotide sequences represented by 5'-ATAGAATTCAAAT-GAACTTTGGCCGACTTCACTG-3' and 5'-ATAAAGCTTCTAACTTTGATGTAAGTT-3', respectively, which were chemically synthesized based on the amino acid sequences nearness to the N- and C-termini of SEQ ID NO: 7, and the mixture solution was brought up to a volume of 100 μl with sterilized distilled water. The solution thus obtained was subjected in a usual manner to PCR reaction of the following three cycles of successive incubations at 94°C for one minute, 43°C for one minute, and 72°C for one minute.

The product obtained by the PCR reaction and "pCR-Script SK (+)", a plasmid vector commercialized by Stratagene Cloning Systems, California, USA, were in a conventional manner figated together using a DNA ligase into a recombinant DNA which was then introduced into "XL-1 Blue MRF'Kan", an Escherichia coli strain commercialized by Stratagene Cloning Systems, California, USA., to obtain a transformant. The transformant was inoculated to L-broth (pH 7.2) containing 50 µg/ml ampicillin, followed by the incubation at 37°C for 18 hours under shaking conditions. The culture was centrifuged to obtain the proliferated transformants which were then treated with a conventional alkali-SDS-method to isolate a recombinant DNA. A portion of the recombinant DNA isolated was analyzed by dideoxyribonucle-otide sequencing, revealing that the recombinant DNA contained restriction sites of Eco RI and Hind III at the 5'- and 3'-termini of SEQ ID NO: 18, respectively, and a DNA containing a methionine codon for initiating polypeptide synthesis and a TAG codon for terminating polypeptide synthesis, which were located in just before and after the N- and C-termini of the amino acid sequence as shown in parallel in SEQ ID NO: 18. The recombinant DNA contained the nucleotide sequences of SEQ ID NOs: 1 to 5. These amino acid sequences were encoded by nucleotides 46-63, 85-102, 394-414, 148-162, and 211-225 in SEQ ID NO: 18.

The remaining portion of the recombinant DNA was in a conventional manner cleaved with restriction enzymes of *Eco* RI and *Hind* II, and the resulting 0.1 µg of an *Eco RI-Hind* III DNA fragments, obtained by using "DNA LIGATION KIT VER 2", a DNA ligation kit commercialized by Takara Shuzo Co., Ltd., Tokyo, Japan, and 10 ng of pKK223-3, a plasmid vector commercialized by Pharmacia LKB Biotechnology AB, Uppsala, Sweden, which had been cleaved with a restriction enzyme were linked together, by incubating at 16°C for 30 min to obtain an autonomously-replicable recombinant DNA, pKGFMH2. Using competent cell method, an *Escherichia coli* Y1090 strain, ATCC 37197, was transformed using the recombinant DNA pKGFMH2, and the resulting transformant, KGFMH2, was inoculated to L-broth (pH 7.2) containing 50 µg/ml ampicillin, and cultured at 37°C for 18 hours under shaking conditions. The culture was centrifuged to collect the proliferated transformants, followed by applying a conventional SDS-alkali method to a portion of the transformants to extract the recombinant DNA pKGFMH2. Dideoxyribonucleotide sequencing analysis revealed that, as shown in FIG. 5, KGFMH2 cDNA containing the nucleotide sequence of SEQ ID NO: 18 was linked to the downstream of the Tac promoter in the recombinant DNA pKGFMH2.

Ampicillin was added to L-broth (pH 7.2), which had been sterilized by autoclaving, to give a concentration of 50 µg/ml, cooled to 37°C, and inoculated with the transformant KGFMH2, followed by the culture at 37°C for 18 hours. Eighteen liters of a fresh preparation of the same culture medium was placed in a 20-f jar fermenter, similarly sterilized as above, admixed with ampicillin, cooled to 37°C, and inoculated with one v/v % of the seed culture obtained in the above, followed by the culture at 37°C for 8 hours under aeration-agitation conditions. The resulting culture was centrifuged to collect the cultured cells which were then suspended in a mixture solution (pH 7.3) containing 150 mM sodium chloride. 16 mM disodium hydrogenphosphate, and 4 mM sodium dihydrogenphosphate, disrupted by ultrasonication, and centrifuged to remove cell disruptant, and this yielded an about two liters of a supernatant.

To an about two liters of the supernatant was added 10 mM phosphate buffer (pH 7.3) containing ammonium sulfate to give a 40% ammonium saturation. The resulting sediment was removed by centrifugation, and the supernatant

was mixed with ammonium sulfate to give an 85% ammonium saturation, allowed to stand at 4°C for 18 hours. and centrifuged at about 8.000 rpm for 30 min to obtain a newly formed sediment. The sediment thus obtained was dissolved in 10 mM phosphate buffer (pH 6.6) containing 1.5 M ammonium sulfate to give a total volume of about 1,300 ml, and the solution was filtered, and fed to a column packed with about 800 ml of "PHENYL SEPHAROSE CL-6B", a gel commercialized by Pharmacia LKB Biotechnology AB. Uppsala, Sweden, followed by washing the column with a fresh preparation of the same buffer and feeding to the column a linear gradient buffer of ammonium sulfate decreasing from 1.5 M to O M in 10 mM phosphate buffer (pH 6.6) at an SV (space velocity) 1.5. Fractions eluted at around 1 M ammonium sulfate were pooled, concentrated using a membrane filter, and dialyzed against 10 mM phosphate buffer (pH 6.5) at 4°C for 18 hours. The dialyzed solution was fed to a column packed with about 55 ml of "DEAE-5PW", a gel commercialized by Pharmacia LKB Biotechnology AB, Uppsala, Sweden, which had been equilibrated with 10 mM phosphate buffer (pH 6.5). The column was washed with a fresh preparation of the same buffer, and fed with a linear gradient buffer of sodium chloride increasing from 0 M to 0.5 M in 10 mM phosphate buffer (pH 6.5) at SV 5.5, followed by collecting fractions eluted at around 0.2 M sodium chloride. Thereafter, the fractions were pooled and concentrated similarly as above up to give an about nine milliliters, followed by dialyzing the concentrate against PBS (phosphate buffered saline) at 4°C for 18 hours, and feeding the dialyzed solution to a column packed with "SUPERDEX 75", a gel commercialized by Pharmacia LKB Biotechnology AB, Uppsala, Sweden, which had been equilibrated with a fresh preparation of the same PBS. The column was fed with a fresh preparation of the same PBS to collect fractions with an IFN-y inducing activity, and the fractions were pooled and concentrated with a membrane filter to obtain a purified mouse IL-18 in a yield of about 350 μg/ℓ culture.

According to the method in Japanese Patent Kokai No. 27,189/96, the purified mouse IL-18 was analyzed and determined for biological activity and physicochemical property as indicated below: Culturing mouse spleen cells, collected by a conventional manner, under different concentrations of the mouse IL-18 resulted in an IFN-γ production depending on the concentrations of the mouse IL-18, and this revealed that the mouse IL-18 has an activity of inducing IFN-γ production by spleen cells as an immunocompetent cell. In accordance with the method as reported by U. K. Laemmli in *Nature*, Vol. 227, pp. 680-685 (1970), the purified human IL-18 was subjected to SDS-PAGE under non-reducing conditions, resulting in a major band with an IFN-γ inducing activity at a position corresponding to 19,000±5,000 daltons. The N-terminal region of the mouse IL-18 contained the amino acid sequence of SEQ ID NO: 19 which corresponded to the N-terminal region of SEQ ID NO: 18.

With reference to Experiment 7, the biological activity of the IL-18 according to the present invention will be described in more detail, and Experiment 8 describes the cytotoxicity of the IL-18:

Experiment 7

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Biological activity

Experiment 7-1

Induction of GM-CSF production

Using a heparinized syringe, blood was collected from a healthy volunteer and diluted two fold with serum-free RPMI 1640 medium (pH 7.4). The diluent was overlaid on a ficoll and centrifuged, and the collected lymphocytes were washed with RPMI 1640 medium (pH 7.4) supplemented with 10 v/v % fetal calf serum, and suspended in a fresh preparation of the same medium to give a cell density of 1 x 10⁶ cells/mI, followed by distributing the cell suspension to a 12-well microplate by two ml/well.

Using RPMI 1640 medium (pH 7.4) supplemented with 10 v/v % fetal calf serum, an IL-18 preparation obtained by the method in Experiment 1 was prepared into a one μ g/ml solution which was then distributed to the above microplate by 20-200 μ l/well. To the microplate was further added a fresh preparation of the same buffer, supplemented with 500 μ l/ml of Concanavalin A, by 10 μ l/well, followed by the incubation at 37°C for 48 hours in a 5 v/v % CO₂ incubator. After completion of the culture, supernatants in each well were sampled by 0.1 ml/well, and determined for GM-CSF content using a conventional enzyme immunoassay. In parallel, a culture system free of IL-18 as a control was provided and treated similarly as above. The data is in Table 1:

Table 1

	Table 1
IL-18* (nM)	GM-CSF yield (pg/ml)
0	510
0.7	2.150

Table 1 (continued)

IL-18* (nM)	GM-CSF yield (pg/ml)
2.8	3,050
5.6	3.950

The results in Table 1 indicate that lymphocytes as an immunocompetent cell produced GM-CSF depending on the concentration of IL-18 when contacted with IL-18 in the presence of Concanavalin A as a cofactor. It was also confirmed that all of the IL-18 preparations and functional equivalents thereof, which were obtained by the methods in Experiments 2 to 5, induced GM-CSF production even when used alone similarly as above. An IL-18 preparation obtained by the method in Experiment 6 was tested in accordance with Experiment 7-1 except that the human lymphocytes used in the experiment were replaced with spleen cells prepared from mouse by a conventional manner, revealing that the IL-18 preparation also induced GM-CSF production.

Experiment 7-2

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Inhibition of osteoclast formation

Experiment 7-2(a)

As reported by T. J. Martin and K. W. Ng *in Journal* of Cellular *Biochemistry*, Vol. 56, pp. 357-366 (1994), it is considered requisite for contacting osteoclastic precursor cells, derived from hematopoietic stem cells, with osteoblasts or bone marrow stromas to generally differentiate osteoclastic precursor cells into mature osteoclasts. As described by G. D. Roodman in Endocrine *Reviews*, Vol. 17, No. 4, pp. 308-332 (1996), it is generally recognized that osteoclasts have characters of multinucleated cells, tartaric acid-resistant acid phosphatase (hereinafter abbreviated as "TRAP") activity, and a calcitonin receptor. In a co-culture system of osteoblasts and bone marrow cells as reported by Nobuyuki UDAGAWA et al., in *Journal of Experimental Medicine*, Vol. 182, pp. 1,461-1,468 (1995), these cells respond to factors such as 1a,25-dihydroxyvitamin D₃, prostaglandin E₂, adrenocortical hormone, interleukin 1, interleukin 6, and interleukin 11, to form osteoclast-like cells (hereinafter may be abbreviated as "OCL"). The formed OCL has characters of osteoclasts *in vivo*. Therefore, the co-culture system well reflects *in vitro* the processes of osteoclast formation in *vivo*. Using this system, experiments for osteoclast formation and osteoclastgenic inhibitory agents can be carried out.

The osteoclastgenic inhibitory activity of the IL-18 according to the present invention was studied using the above co-culture system. The osteoblasts used in this experiment were prepared in a conventional manner by treating a newborn mouse calvaria with 0.1 w/v % collagenase commercialized by Worthington Biochemical Co.. Freefold, Australia, and 0.2 w/v % dispase commercialized by Godo Shusei Co., Ltd., Tokyo, Japan. The bone marrow cells were prepared from a mature mouse in a conventional manner. As a negative control, 2 x 104 cells of a primary cell culture of osteoblasts and 5 x 105 cells of bone marrow cells were co-cultured in each well of a 48-well microplate containing 0.4 ml/well of α-MEM medium supplemented with 10 v/v % fetal calf serum (hereinafter designated as "Medium" throughout Experiment 4-2) at 37°C for seven days in a 5 v/v % CO₂ incubator. As a positive control, the above twotypes of cells were co-cultured similarly as in the negative control except that they were cultured in other wells containing 10⁻⁸M of 1α,25-dihydroxyvitamin D₃ commercialized by Wako Pure Chemicals, Tokyo, Japan, and 10⁻⁷M of prostaglandin E2 commercialized by Sigma Chemical Company, Missouri, USA. The aforesaid two-types of cells were cocultured similarly as in the positive control except that they were cultured in other wells containing 1α,25-dihydroxyvitamin D₃ commercialized by Wako Pure Chemicals, Tokyo, Japan, and prostaglandin E₂ commercialized by Sigma Chemical Company, Missouri, USA., in the same concentrations as used in the positive control, and a concentration of 0.01-10 ng/ml of an IL-18 preparation prepared by the method in Experiment 6. In every co-culture system, the media in each well were replaced with fresh preparations of the same media used in the co-culture systems on the 3rd day after the initiation of each culture. According to the method by Nobuyuki UDAGAWA in Journal of Experimental Medicine, Vol. 182, pp. 1,461-1,468 (1995), the cells on the 6th day after the initiation of each culture were fixed and stained based on TRAP activity, followed by counting the stained cells (hereinafter called "TRAP-positive cells") per well. Throughout Experiment 4-2, quadruplet wells under the same conditions were provided for each co-culture system. and the mean value for the TRAP-positive cells per well in each system was calculated. The results are in Table 2:

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25		2
30		Table
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IL-18 (ng/ml)	Osteoclastgenic formation factor ¹ 1	Number of TRAP-positive cells per well 2
0		2
0	+	110
0.01	+	114
0.1	+	111
0.5	+	106
1	+	63
2	+	29
Ţ.	+	12
80	+	2
10	+	

The symbols of "+" and "-" show co-culture systems with and without $10^{-6}M$ la,25-dihydroxyvitamin D_3 and $10^{-7}M$ prostaglandin E_2 , respectively. It shows a mean value of the data from quadruplet wells cultured under the same conditions. Note: 1:

*2:

As shown in Table 2, the formation of TRAP-positive cells was not substantially observed in the negative control, but the distinct formation was observed in the positive control. In the co-culture systems, i.e., the positive control supplemented additionally with IL-18, the formation of TRAP-positive cells was inhibited depending on the concentration of IL-18, and the maximum inhibition, i.e., a level equal to that in the negative control, was found at eight ng/ml or more of IL-18. These data strongly indicates that IL-18 has a concrete activity of inhibiting OCL formation in *vitro* and also inhibits osteoclast formation.

Experiment 7-2(b)

As described hereinbefore, it was confirmed that there exist factors that induce the formation of osteoclast-like cells in the co-culture systems used throughout Experiment 7-2. Therefore, in this Experiment 7-2(b), it was studied whether the inhibitory activity of IL-18 on osteoclast formation observed in Experiment 7-2(a) was specific to some factors or not; the osteoclast-like cells were cultured by the same method as used in the negative control in Experiment 7-2(a) except for using a medium supplemented with 10^{-8} M 1α ,25-dihydroxyvitamin D_3 , 10^{-7} M prostaglandin E_2 , 200 ng/ml parathyroid hormone, 100 ng/ml interleukin 1, or 20 ng/ml interleukin 11. These culture systems were for positive controls. In parallel, the cells were cultured in other wells by the same method used in the positive controls except for using a medium containing 10 ng/ml of an IL-18 preparation obtained by the method in Experiment 6, in addition to any one of the above factors at the same concentration. After completion of the cultures, TRAP-positive cells in each well were counted, and the numbers were compared similarly as in Experiment 7-2(a). The results are in Table 3:

Table 3

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Osteocla (Osteoclast formation factor'l (concentration)	1L-18*2	Number of TRAP-positive cells per well'3
c	(Mg-U L)	1	94
້	(1.01)	+	Е
<u>م</u>	(M2-01)	t	77
, OL2	(1, 0, 1)	+	3
Ħ	(=/ 50 (000)	1	63
•	(4) (B) (2)	+	3
11,-11	([m/5u 001) [1-11])		84
		+	3
1	([m/bd/06/ [-11	•	71
f 1	(4 /B::) 1 /	+	3

prostaglandin E_2 , parathyroid hormone, interleukin-11, and interleukin-1 which were added to wells to give the concentrations as indicated in parentheses. The symbol "+" means that IL-18 was added to a well to give a concentration of 10 ng/ml, and the symbol "-" means that IL-18 was not added to. It shows a mean value of the data from quadruplet wells cultured under the same $D_{\rm j},~PGE_{\rm 2},~PTH,~IL-11,~and~IL-1$ are respectively 1lpha,25-dihydroxyvitamin $D_{\rm j},$ 2: .. 3 Note:

conditions.

As shown in Table 3. a distinct formation of TRAP-positive cells was observed in every positive control, but the formation was almost completely inhibited in the presence of IL-18. This strongly indicates that IL-18 has a wide and general activity of inhibiting osteoclast formation independently of osteoclast-formation-related factors.

Experiment 7-2(c)

It was studied whether the osteoclastgenic inhibition by IL-18, confirmed in Experiments 7-2(a) and 7-2(b), was caused by the action of the IL-18-induced GM-CSF. For positive and negative controls, the same co-culture systems employed in Experiment 7-2(a) were used. Using other wells, the co-culture of osteoblasts and bone marrow cells was carried out similarly as the method used for the positive controls except for using a medium supplemented with 1α , 25-dihydroxyvitamin D_3 and prostaglandin E_2 at the same concentrations used in the positive control, and with (i) 10 μ g/ml of an anti-mouse GM-CSF polyclonal antibody commercialized by R&D Systems, Minnesota, USA, (ii) 10 μ g/ml of an IL-18 preparation obtained by the method in Experiment 6, (iii) (ii) plus 10 μ g/ml of an anti-mouse GM-CSF commercialized by R&D Systems, Minnesota, USA, or (v) (iv) plus 10 μ g/ml of an anti-mouse GM-CSF polyclonal antibody. After completion of the culture, TRAP-positive cells in each well were counted, and the numbers were compared similarly as in Experiment 7-2(a). The data is shown in Table 4 where the symbols "i" to "v" coincide with those used in the co-culture systems other than the control systems.

	i		! !	l	l	l	l	ı	1
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10		Number of TRAP-positive cells per well'6	3	122	112	က	111	4	106
15		Number of cells		. 1	1		1		-
20		Anti-GM-CSF antibody*5							
25	4	Anti-	ŧ	ŧ	+	t	+	ı	+
30	Table 4	GM-CSF*4	ţ	ŧ	ı	ţ	t	+	+
35		nic IL-18*3	ſ	1	1	+	+	ŧ	ı
40		stgenic							
45		Osteoclastge factor'2	•	+	+	+	+	+	+
50		Culture system¹l	Z	Ω,	į	11	111	iv	>
55									

controls, respectively, and the symbols "i" to "v" correspond "1; where the symbols "N" and "P" mean negative and positive to those in the five types co-culture systems used. Note:

where the symbol "+" means that $1\alpha,25$ -dihydroxyvitamin D_3 and prostaglandin E_2 were respectively added to a well to give respective concentrations of $10^{-6}M$ and $10^{-7}M$, and the symbol *2;

concentration of 10 ng/ml, and the symbol "-" means that IL-18 "-" means that these compounds were not added to. The symbol "+" means that IL-18 was added to a well to give a was not added to. . Ω,

concentration of 0.1 ng/ml, and the symbol "-" means that GM-CSF The symbol "+" means that GM-CSF was added to a well to give a was not added to. • 4

The symbol "+" means that an anti-GM-CSF polyclonal antibody was added to a well to give a concentration of $10~\mu g/ml$, and the symbol "-" means that the polyclonal antibody was not added to. . 5

As shown in Table 4, the formation of TRAP-positive cells was almost completely inhibited by IL-18, cf., the co-culture system (ii), but the inhibition was almost completely inhibited by the addition of the anti-mouse polyclonal antibody, cf., the co-culture system (iii). Mouse GM-CSF exhibited an activity of inhibiting the formation of TRAP-positive cells similar to IL-18, cf., the co-culture system (iv), and the inhibition was almost completely inhibited by the addition of the anti-mouse GM-CSF polyclonal antibody, cf., the co-culture system (v). The sole use of the anti-mouse GM-CSF polyclonal antibody gave no influence on the formation of TRAP-positive cells, cf., the co-culture system (i). These data strongly indicates that the osteoclastgenic inhibition by IL-18 was due to the action of the IL-18-induced GM-CSF.

Experiment 8

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Acute toxicity test

Eight-week-old mice were in a conventional manner injected percutaneously, orally, or intraperitoneally with either of IL-18 preparations obtained by the methods in Experiments 1 to 6. The results showed that these IL-18 preparations had an LD₅₀ of about one mg/kg or more in mice independent of the route of administration. The data evidences that IL-18 can be incorporated into pharmaceuticals for warm-blooded animals in general and including humans without causing no serious side effects.

As described in *Nikkei Biotechnology Annual Report 1996*, pp. 498-499 (1995), published by Nikkei BP Publisher, Tokyo, Japan (1995), the IL-18-induced GM-CSF has not yet been clinically used in Japan, but applied clinically in USA and Europe. The fact would show that IL-18 has substantially no serious side effects. These facts indicate that the osteoclastgenic inhibitory agent according to the present invention can be successively administered to warm-blooded animals in general and including humans to induce osteoclast formation and exert a satisfactory therapeutic and/or prophylactic effect on osteoclast-related diseases without causing serious side effects.

The following Examples describe the present osteoclastgenic inhibitory agent according to the present invention:

Example 1

Liquid

Either of IL-18 preparations, obtained by the methods in Experiments 1 to 6, was dissolved in physiological saline containing one w/v % human serum albumin as a stabilizer to give a concentration of two mg/ml of the IL-18 preparation. The resulting solutions were in a conventional manner membrane filtered for sterilization into liquids.

The liquids have a satisfactory stability and can be arbitrarily used as ingredients for cell culture and agents in the form of an injection, ophthalmic solution, or collunarium for regulating bone resorption and for osteoclast-related diseases, directed to treat and/or prevent hypercalcemia, osteoclastoma, osteoporosis, etc.

Example 2

Dry agent

Fifty milligrams of either of IL-18 preparations, obtained by the methods in Experiments 1 to 6, was dissolved in 100 ml of physiological saline containing one w/v % purified gelatin as a stabilizer. The solutions thus obtained were in a conventional manner membrane filtered for sterilization, distributed to vials by one milliliter, lyophilized, and sealed with caps.

The products have a satisfactory stability and can be arbitrarily used as ingredients for cell culture and agents in the form of a dry injection for regulating bone resorption and for osteoclast-related diseases, directed to treat and/or prevent hypercalcemia, osteoclastoma, osteoporosis, etc.

Example 3

Dry agent

Fifty milligrams of either of IL-18 preparations, obtained by the methods in Experiments 1 to 6, was dissolved in 100 ml of physiological saline containing one w/v % trehalose as a stabilizer. The solutions were in a conventional manner membrane filtered for sterilization, distributed to vials by one milliliter, lyophilized, and sealed with caps.

The products have a satisfactory stability and can be arbitrarily used as ingredients for cell culture and agents in the form of a dry injection for regulating bone resorption and for osteoclast-related diseases, directed to treat and/or prevent hypercalcemia, osteoclastoma, osteoporosis, etc.

Example 4

Ointment

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"HIVIS WAKO GEL® 104", a carboxyvinylpolymer commercialized by Wako Pure Chemical Industries, Ltd., Tokyo, Japan, and a high-purity trehalose were dissolved in a sterilized distilled water to give respective concentrations of 1.4 w/w % and 2.0 w/w %, and the solution was mixed to homogeneity with either of IL-18 preparations obtained by the methods in Experiments 1 to 6, and adjusted to pH 7.2 to obtain a paste containing about one mg of an IL-18 preparation per g of the product.

Each product thus obtained has a satisfactory spreadability and stability and can be arbitrarily used as an agent in the form of an ointment for regulating bone resorption and for osteoclast-related diseases, directed to treat and/or prevent hypercalcemia, osteoclastoma, osteoporosis, etc.

Example 5

Tablet

"FINETOSE®", an anhydrous crystalline α-maltose powder commercialized by Hayashibara Biochemical Laboratories, Inc., Okayama, Japan, was mixed to homogeneity with either of IL-18 preparations, obtained by the methods in Experiments 1 to 6, and "LUMIN" or 1-1'-1"-trihepthyl-11-chinolyl(4)•4•4'-penthamethinchynocyanine-1,1"-dijodide. The mixtures were in a conventional manner tabletted to obtain tablets, about 200 mg weight each, containing an about two milligrams of either of the IL-18 preparations and an about two milligrams of LUMIN per tablet.

The products have a satisfactory swallowability, stability, and cell-activating activity and can be arbitrarily used as agents in the form of a tablet for regulating bone resorption and for osteoclast-related diseases, directed to treat and/or prevent hypercalcemia, osteoclastoma, osteoporosis, etc.

As described above, the osteoclastgenic inhibitory agent according to the present invention effectively inhibits osteoclast formation. Therefore, the agent can be arbitrarily used as an ingredient for cell culture and agents for regulating bone resorption and for osteoclast-related diseases, directed to treat and/or prevent hypercalcemia, osteoclastoma, osteoporosis, etc.

Thus the present invention with these useful activities and functions is a significant invention that would greatly contribute to this field.

While there has been described what is at present considered to be the preferred embodiments of the invention, it will be understood the various modifications may be made therein, and it is intended to cover in the appended claims all such modifications as fall within the true spirits and scope of the invention.

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Annex to the description

5 .	SEQUENCE LISTING
	(1) INFORMATION FOR SEQ ID NO: 1:
10	<pre>(i)SEQUENCE CHARACTERISTICS: (A)LENGTH: 6 amino acids (B)TYPE: amino acid (D)TOPOLOGY: linear</pre>
	(ii)MOLECULE TYPE: peptide
15	(v)FRAGMENT TYPE: internal fragment
	(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 1:
20	Asn Asp Gln Val Leu Phe 1 5
25	(2) INFORMATION FOR SEQ ID NO: 2: (i)SEQUENCE CHARACTERISTICS: (A)LENGTH: 6 amino acids (B)TYPE: amino acid (D)TOPOLOGY: linear
	(ii) MOLECULE TYPE: internal fragment
30	(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 2:
	Phe Glu Asp Met Thr Asp 1 5
35	<pre>(3) INFORMATION FOR SEQ ID NO: 3: (i)SEQUENCE CHARACTERISTICS: (A)LENGTH: 7 amino acids (B)TYPE: amino acid (D)TOPOLOGY: linear</pre>
40	(ii)MOLECULE TYPE: peptide
	(v)FRAGMENT TYPE: internal fragment
	(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 3:
45	Phe Lys Leu Ile Leu Lys Lys 1 5
	(4) INFORMATION FOR SEQ ID NO: 4:
50	(i)SEQUENCE CHARACTERISTICS: (A)LENGTH: 5 amino acids (B)TYPE: amino acid (D)TOPOLOGY: linear
55	(ii)MOLECULE TYPE: internal fragment

```
(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 4:
5
         Met Tyr Lys Asp Ser
         (5)
               INFORMATION FOR SEO ID NO: 5:
               (i) SEQUENCE CHARACTERISTICS:
10
                    (A)LENGTH: 5 amino acids
                    (B)TYPE: amino acid
                    (D)TOPOLOGY: linear
               (ii) MOLECULE TYPE: internal fragment
15
               (xi)SEQUENCE DESCRIPTION: SEQ ID NO: 5:
         Ser Thr Leu Ser Cys
20
         (6)
               INFORMATION FOR SEQ ID NO: 6:
               (i) SEQUENCE CHARACTERISTICS:
                    (A)LENGTH: 157 amino acids
25
                    (B)TYPE: amino acid
                    (D)TOPOLOGY: linear
               (ii) MOLECULE TYPE: peptide
30
               (xi)SEQUENCE DESCRIPTION: SEQ ID NO: 6:
         Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn
                                               10
         Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp
35
                      20
                                           25
         Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile
                                                           45
         Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile
                                                       60
         Ser Val Lys Cys Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile
40
                                                   75
         Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys
                          85
                                               90
                                                                    95
         Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys
                      100
45
                                           105
         Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu
                                      120
                                                           125
         Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu
                                  135
         Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp
50
         145
                              150
                                                   155
         (7)
              INFORMATION FOR SEQ ID NO: 7:
               (i) SEQUENCE CHARACTERISTICS:
55
                    (A)LENGTH: 157 amino acids
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			•	3)TYF O)TOF													
5		(ii	L)MOI	LECUI	LE TY	PE:	pept	ide									
		(x))SEÇ	QUENC	CE DE	ESCRI	PTIC	ON: S	SEQ 1	D NO): 7:	:					
10	1	Phe	•		5					10					15		
	-	Gln		20					25					30			
		Asp	35	_				40					45				
15	_	Met 50	_				55					60					
	65	Lys	_			70					75					80	
20		Phe			85					90					95		
20		Leu		100					105					110			
		Glu	115					120					125				
25	_	Asp 130					135					140		ASII	GIY	ASP	
	Lys 145	Ser	vai	Met	Pne	150	Leu	TILL	ASII	Leu	155	GIII	Ser	,			
	(8)	INFO	RMAT	ION	FOR :	SEQ	ID N	0: 8	:								
30		(i		UENCI A)LEI					-								
			(BÍTY! C)STI	PE:	nucl	eic a	acid							-		
35				D)TO													
		(i	i)MO	LECU	LE T	YPE:	CDN	A									
		(v	• .	IGIN A)OR				n									
40			•	G)CE		YPE:	liv	er									
		(i .	(ATUR A)NA	ME/K				ide								
			•	B)LO					HOD:	E							
45		ж)	i)SE	QUEN	CE D	ESCR	IPTI	ON:	SEQ	ID N	0: 8	:					
T	AC T	TT GO	GC A	AG C	rt G	AA TO	CT A	AA T	ra To	CA GT	C A	CA AC	SA A	AT TO	rg A	AT	48
1	-	ne G. AA G'	_	5					10)				15	5		96
G A	sp G	AA G	al L	eu Pi	he I	le As	sp G	In G	ly A	sn A	g Pi	co Le	eu Pl	he G	Lu A	sp	50
A	TG A	CT G	20 AT T		AC TO	GT A	GA G			CA CO	CC CC	GG AC			rt A	ГT	144

		THE	33					40					45				
	ATA	AGT	ATG	TAT	AAA	GAT	AGC	CAG	CCT	AGA	GGT	ATG	CCT	GTA	АСТ	ልጥሮ	100
5	rre	50	met	туг	rys	Asp	Ser 55	Gln	Pro	Arg	Gly	Met 60	Ala	Val	Thr	Ile	192
	TCT	GTG	AAG	TGT	GAG	AAA	ATT	TCA	ACT	CTC	TCC	TGT	GAG	AAC	ΔΔΔ	ል ጥጥ	240
	Ser 65	vaı	rys	Cys	Glu	Lys 70	Ile	Ser	Thr	Leu	Ser 75	Cys	Glu	Asn	rās	Ile	240
10	ATT	TCC	TTT	AAG	GAA	ATG	AAT	CCT	CCT	GAT	AAC	ATC	AAG	GAT	ACA	888	288
	ite	ser	Pne	гĀЗ	85	Met	Asn	Pro	Pro	Asp 90	Asn	Ile	Lys	Asp	Thr	Lys	200
	AGT	GAC	ATC	ATA	TTC	TTT	CAG	AGA	AGT	GTC	CCA	GGA	CAT	GAT	AAT	AAG	336
15	Ser	ASp	rre	100	Pne	Pne	GIn	Arg	Ser	Val	Pro	Gly	His	Asp	Asn	Lys	330
,,	ATG	CAA	TTT	GAA	TCT	TCA	TCA	TAC	GAA	GGA	TAC	TTT	CTA	GCT	TGT	GAA	384
	met	GIN	115	GIU	Ser	Ser	Ser	Tyr 120	Glu	Gly	Tyr	Phe	Leu 125	Ala	Cys	Glu	
	AAA	GAG	AGA	GAC	CTT	TTT	AAA	CTC	ATT	TTG	AAA	AAA	GAG	GAT	GAA	TTG	432
20		Glu 130					135					140		Asp	Glu	Leu	
	GGG	GAT	AGA	TCT	ATA	ATG	TTC	ACT	GTT	CAA	AAC	GAA	GAC				471
	145	Asp	rry	261	TIE	150	Pne	Thr	vaI	GIn	155	Glu	Asp				
25	(9)	INE	FORMA	OITA	V FOF	R SEC) ID	NO:	9:								
		(i)	SEQU	JENCE	E CHA	RACI	ERIS	TICS	5:								
			(E	B)TYI	NGTH:	mino	aci	.đ	cids								
30			(L	7 1 1 02	POLOG	71; 1	ınea	ır									
30		(ii	L)MOI	LECUI	LE TY	PE:	pept	ide									
		(v)	FRAG	MENT	TYF	E: N	-ter	mina	l fr	agme	nt					••	
<i>35</i>		(xi)SEQ	UENC	E DE	SCRI	PTIO	N: S	EQ I	D NO	: 9:						
	Met 1	Tyr	Phe	Gly	Lys 5	Leu	Glu	Ser	Lys	Leu 10	Ser						
40	(10)	INF	ORMA	OITA	FOR	SEQ	ID	ΝО:	10:								
		(i)	(A (B) LEN	CHA GTH: PE: a POLOG	10 mino	amin aci	o ac d									
4 5		(ii)MOL	ECUL	Е ТҮ	PE:	pept	ide									
		(v)	FRAG	MENT	TYP	E: C	-ter	mina	l fr	agme	nt						
		(xi)SEQ	UENC	E DE	SCRI	PTIO	N: S	EQ I	D NO	: 10	:					
50	Ser 1	Ile	Met	Phe	Thr 5	Val	Gln .	Asn		Asp 10							

	(11) INFORMATION FOR SEQ ID NO: 11:
5	(i)SEQUENCE CHARACTERISTICS:(A)LENGTH: 13 amino acids(B)TYPE: amino acid(D)TOPOLOGY: linear
10	(ii)MOLECULE TYPE: peptide
	(v)FRAGMENT TYPE: N-terminal fragment
	(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 11:
15	Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg 1 5 10
	(12) INFORMATION FOR SEQ ID NO: 12:
20	<pre>(i)SEQUENCE CHARACTERISTICS: (A)LENGTH: 14 amino acids (B)TYPE: amino acid (D)TOPOLOGY: linear</pre>
	(ii)MOLECULE TYPE: peptide
25	(v)FRAGMENT TYPE: internal fragment
	(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 12:
30	Thr Ile Phe Ile Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg 1 5 10
	(13) INFORMATION FOR SEQ ID NO: 13:
35	(i)SEQUENCE CHARACTERISTICS:(A)LENGTH: 17 amino acids(B)TYPE: amino acid(D)TOPOLOGY: linear
	(ii)MOLECULE TYPE: peptide
40	(v)FRAGMENT TYPE: internal fragment
	(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 13:
4 5	Ile Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys 1 10 15
	(14)INFORMATION FOR SEQ ID NO: 14:
50	(i)SEQUENCE CHARACTERISTICS: (A)LENGTH: 471 base pairs (B)TYPE: nucleic acid (C)STRANDEDNESS: double (D)TOPOLOGY: linear

		(ii)MOI	ECUI	E TY	PE:	CDNA	.								• .	
5		(1x	(E) NAM	E/KE	N: 1	nat p 47	1		s							
		(xi					PTIC): 14	:					
10																	
	TAC	TTT	GGC	AAG	CTT	GAA	TCT	AAA	TTA	TCA	GTC	ATA	AGA	AAT	TTG	AAT	48
	l yr	Pne	Gry	ьys	seu 5	Gru	ser	гĀŻ	Leu	ser 10	vaı	TTE	Arg	Asn		Asn	
		CAA	GTT	CTC	TTC	ATT	GAC	CAA	GGA		CGG	ССТ	CTA	ጥጥጥ	15 GAA	CAT	96
15	Asp	Gln	Val	Leu 20	Phe	Ile	Asp	Gln	Gly 25	Asn	Arg	Pro	Leu	Phe 30	Glu	Asp	30
	ATG	ACT	GAT	TCT	GAC	TCT	AGA	GAT	AAT	GCA	CCC	CGG	ACC	ATA	TTT	ATT	144
	•		35					40					Thr 45				
20	ATA	AGT	ATG	TAT	AAA	GAT	AGC	CAG	CCT	AGA	GGT	ATG	GCT	GTA	ACT	ATC	192
		50					55					60	Ala				
	Ser	Val	Lve	Ser	Glu	LVC	Tla	Ser	Thr	Leu	Sor	Ala	GAG Glu	AAC	AAA	ATT	240
	65	142	~, ~		014	70	110	561	1111	neu	75	VIG	GIU	ASII	ьуѕ	80	
25	ATT	TCC	TTT	AAG	GAA	ATG	AAT	CCT	CCT	GAT	AAC	ATC	AAG	GAT	ACA	AAA	288
	Ile	Ser	Phe	Lys	Glu 85	Met	Asn	Pro	Pro	Asp 90	Asn	Ile	Lys	Asp	Thr 95	Lys	200
	AGT	GAC	ATC	ATA	TTC	TTT	CAG	AGA	AGT	GTC	CCA	GGA	CAT	GAT	AAT	AAG	336
30				100					105				His	110		_	
00	Mot	Gln	Pho	GAA	TCT	TCA	TCA	TAC	GAA	GGA	TAC	TTT	CTA	GCT	TGT	GAA	384
			115					120					Leu 125		_		
	Lvs	Glu	Ara	Asn	Len	Phe	Lve	Len	TIA	LOU	Lyc	Luc	GAG Glu	GAT	GAA	TTG	432
35	2,3	130	y	пор	Dea	1110	135	LEU	116	Беа	цуз	140	GIU	ASD	GIU	rea	
33	GGG	GAT	AGA	TCT	ATA	ATG		ACT	GTT	CAA	AAC	GAA	GAC				471
	Gly 145	Asp	Arg	Ser	Ile	Met 150	Phe	Thr	Val	Gln	Asn 155	Glu	Asp				
40	(15) INI	FORM	OITA	V FO	R SE	DI C	NO:	15:								
		(i	(1	A)LEI	NGTH:	: 10 amino	reris amin ac: lines	no ad id									
45		(i:	i)MO	LECUI	LE T	YPE:	pep	tide									
							V-te		al fi	cagme	ent						
50		(x :	i)SE(QUEN	CE DI	ESCR	[PTIC	ON: S	SEQ 1	ED NO	D: 15	5:					
50	Tyr 1	Phe	Gly	Lys	Leu 5	Glu	Ser	Lys	Leu	Ser 10							
										-							

	(16)	INFO	RMAI	NOI	FOR	SEQ	ID N	10: 1	6:						-		
5		(1)	(E	A)LEN B)TYE C)STE	NGTH: PE: r RANDE	: 471 nucle	bas ic a SS: c	se pa acid doub]	airs								
10		(ii	L)MOI	LECUI	E T	PE:	CDN	4									
·		(i)	(E	1) NAN 3) LOC	ME/KI	ON: 1	L47	pepti 71 METI		s							
15		(x:	i)se(QUENC	CE DE	ESCR	(PTI	ON: S	SEQ 1	נס אס	D: 16	5:					
			GGC Gly														48
20	GAC		GTT Val												GAA		96
			GAT Asp 35														144
25			ATG Met					CAG					GCT				192
30		GTG	AAG Lys														240
	Ile	Ser	TTT Phe	Lys	Glu 85	Met	Asn	Pro	Pro	Asp 90	Asn	Ile	Lys	Asp	Thr 95	Lys	288
35	Ser	Asp	ATC Ile	11e 100	Phe	Phe	Gln	Arg	Ser 105	Val	Pro	Gly	His	Asp 110	Asn	Lys	336
	Met	Gln	TTT Phe 115	Glu	Ser	Ser	Ser	Tyr 120	Glu	Gly	Tyr	Phe	Leu 125	Ala	Ser	Glu	384
40	Lys	Glu 130	AGA Arg	Asp	Leu	Phe	Lys 135	Leu	Ile	Leu	Lys	Lys 140	Glu	GAT Asp	GAA Glu	TTG Leu	432
		Asp	AGA Arg														471
45	(17)INF	ORMA	TION	FOR	SEQ	ID	NO:	17:								
		(1		A)LE	NGTH	: 11	464	STIC base acid	pai	rs							
50			(C)ST	RAND		ss:	doub									

	(ii)MOLECULE TYPE: genomic DNA	
5	<pre>(vi)ORIGINAL SOURCE: (A)ORGANISM: human (G)CELL TYPE: placenta</pre>	
10	<pre>(ix)FEATURE: (A)NAME/KEY: 5 UTR (B)LOCATION: 13 (C)IDENTIFICATION METHOD: E (A)NAME/KEY: leader peptide (B)LOCATION: 482 (C)IDENTIFICATION METHOD: S</pre>	
15	(A)NAME/KEY: intron (B)LOCATION: 831453 (C)IDENTIFICATION METHOD: E (A)NAME/KEY: leader peptide	
20	(B)LOCATION: 14541465 (C)IDENTIFICATION METHOD: S (A)NAME/KEY: Intron (B)LOCATION: 14664848 (C)IDENTIFICATION METHOD: E (A)NAME/KEY: leader peptide	
?5	(B)LOCATION: 48494865 (C)IDENTIFICATION METHOD: S (A)NAME/KEY: mat peptide (B)LOCATION: 48664983 (C)IDENTIFICATION METHOD: S (A)NAME/KEY: intron	
30	(B)LOCATION: 49846317 (C)IDENTIFICATION METHOD: E (A)NAME/KEY: mat peptide (B)LOCATION: 63186451 (C)IDENTIFICATION METHOD: S	
35	(A)NAME/KEY: intron (B)LOCATION: 645211224 (C)IDENTIFICATION METHOD: E (A)NAME/KEY: mat peptide (B)LOCATION: 1122511443 (C)IDENTIFICATION METHOD: S (A)NAME/KEY: 3 UTR	
10	(B)LOCATION: 1144411464 (C)IDENTIFICATION METHOD: E	
	(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 17:	
15	AAG ATG GCT GCT GAA CCA GTA GAA GAC AAT TGC ATC AAC TTT GTG GCA Met Ala Ala Glu Pro Val Glu Asp Asn Cys Ile Asn Phe Val Ala -35 -30 -25	48
	ATG AAA TTT ATT GAC AAT ACG CTT TAC TTT ATA G GTAAGG CTAATGCCAT Met Lys Phe Ile Asp Asn Thr Leu Tyr Phe Ile Ala -20 -15 -10	98
50	AGAACAAATA CCAGGTTCAG ATAAATCTAT TCAATTAGAA AAGATGTTGT GAGGTGAACT ATTAAGTGAC TCTTTGTGTC ACCAAATTTC ACTGTAATAT TAATGGCTCT TAAAAAAATA GTGGACCTCT AGAAATTAAC CACAACATGT CCAAGGTCTC AGCACCTTGT CACACCACGT GTCCTGGCAC TTTAATCAGC AGTAGCTCAC TCTCCAGTTG GCAGTAAGTG CACATCATGA	158 218 278 338

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                                                                              398
      GTACAAAACT GGGTGCATTC AGGAAATACA ATTTCCCAAA GCAAATTGGC AAATTATGTA
                                                                              458
      AGAGATTCTC TAAATTTAGA GTTCCGTGAA TTACACCATT TTATGTAAAT ATGTTTGACA
                                                                              518
      AGTAAAAATT GATTCTTTTT TTTTTTTCT GTTGCCCAGG CTGGAGTGCA GTGGCACAAT
                                                                              578
      CTCTGCTCAC TGCAACCTCC ACCTCCTGGG TTCAAGCAAT TCTCCTGCCT CAGCCTTCTG
                                                                              638
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                                                                              698
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                                                                              758
      CCTGGCTCGG GCTCCCAAAG TGCTGGGATT ACAGGCATGA ACCACCACAC ATGGCCTAAA
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                                                                              878
10
      ATTTGAAACC TTCATTTAAA AGCCTGAGCA ACAAAGTGAG ACCCCATCTC TACAAAAAAC
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      TGCAAAATAT CCTGTGGACA CCTCCTACCT TCTGTGGAGG CTGAAGCAGG AGGATCACTT
                                                                              998
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                                                                             1178
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                                                                             1470
                                               Ala Glu Asp Asp Glu
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                                                -10
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       ATGTGGACTC AGTAGCACAG CTTTGGAATG AAGATGATCA TAAGAGATAC AAAGAAGAAC
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       CTCTAGCAAA AGATGCTTCT CTATGCCTTA AAAAATTCTC CAGCTCTTAG AATCTACAAA
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                                                                             1770
25
      1830
                                                                             1890
                                                                             1950
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       GTAGAGTAGG AGTAGGAGAC TGGTGAGAGG AGCTAGAGTG ATAAACAGGG TGTAGAGCAA
                                                                             2070
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                                                                             2250
                                                                             2310
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                                                                             2730
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       TGGAAAGGAG AAGAAGTAG AAAAGATGAT GCCTACATTT TTCACTTAGG CAATTTGTAC
       CATTCAGTGA AATAGGGAAC ACAGGAGGAA GAGCAGGTTT TGGTGTATAC AAAGAGGAGG
                                                                              2910
                                                                              2970
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       CACAAACTCT TCTACATGTG GTTCTGAGTT CAGGACACAG ATTTGGGCTG GAGATAGAGA
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       TTAAAGGATG CAGTAGAAAG AAGCTAATAA ACAACAGAGA GCAGACTAAC CAAAAGGGGA
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       CCTAAGCTGC TTTTTCTAGT TAGTGATATA TATGGACATC TCTCCATGGC AACGAGTAAT
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                                                                                  4410
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                                                                                   4710
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CTAAAATATA TAGCATACTT ATTTGTCAAT TAACAAAGAA ACTATGTATT TTTAAATGAG
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                                                                                  4830
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                                                                                  4880
                             Glu Asn Leu Glu Ser Asp Tyr Phe Gly Lys Leu
                                   -5
       GAA TCT AAA TTA TCA GTC ATA AGA AAT TTG AAT GAC CAA GTT CTC TTC
                                                                                  4928
25
       Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn Asp Gln Val Leu Phe
                         10
                                               15
       ATT GAC CAA GGA AAT CGG CCT CTA TTT GAA GAT ATG ACT GAT TCT GAC
                                                                                  4976
       Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp Met Thr Asp Ser Asp
                                          30
                                                                 35
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                   GTATTTTT TTAATTCGCA AACATAGAAA TGACTAGCTA CTTCTTCCCA
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       Cys Arg Asp
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                                                                                  5212
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       CTGTAGTACA CTGTGATCGT ACCTGTGAAT AGCCACTGCA CTCCAGCCTG GGTGATATAC
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       AGACCTTGTC TCTAAAATTA AAAAAAAAA AAAAAAAAC CTTAGGAAAG GAAATTGATC
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       AGTGAATGTG CATTCTTTAA AAATACTGAA TACTTACCTT AACATATATT TTAAATATTT
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                                                                                  5932
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       TCAGCCTCCC AAACAAACAA ACAACCCCAC AGTTTAATAT GTGTTACAAC ACACATGCTG
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50
                                                                                  6292
       TTCTCATTTA TTATATTTAT TTCAG AT AAT GCA CCC CGG ACC ATA TTT ATT
                                      Asp Asn Ala Pro Arg Thr Ile Phe Ile
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			40		45	·	
			40		45		
	ATA AGT ATG	TAT AAA GA	T AGC CAG	CCT AGA GGT	ATG GCT GTA	ACT ATC	6391
		Tyr Lys As		Pro Arg Gly		Thr IIe	
5	50		55		60		
	TCT GTG AAG	TGT GAG AA	A ATT TCA	ACT CTC TCC	TGT GAG AAC	AAA ATT	6439
	Ser Val Lys			Thr Leu Ser	Cys Glu Asr		
	65	70		75		80	
			ACTGAGCCTT	ACTTTGTTTT	CAATCATGTT .	AATATAATCA	6496
45	Ile Ser Phe	Lys					
10	ATATAATTAG	AAATATAACA	TTATTTCTAA	TGTTAATATA	AGTAATGTAA	TTAGAAAACT	6556
	CAAATATCCT	CAGACCAÁCC	TTTTGTCTAG	AACAGAAATA	ACAAGAAGCA	GAGAACCATT	6616
	AAAGTGAATA	CTTACTAAAA	ATTATCAAAC	TCTTTACCTA	TTGTGATAAT	GATGGTTTTT	6676
	CTGAGCCTGT	CACAGGGGAA	GAGGAGATAC	AACACTTGTT	TTATGACCTG	CATCTCCTGA	6736
	ACAATCAGTC	TTTATACAAA	TAATAATGTA	GAATACATAT	GTGAGTTATA	CATTTAAGAA	6796
15	TAACATGTGA	CTTTCCAGAA	TGAGTTCTGC	TATGAAGAAT	GAAGCTAATT	ATCCTTCTAT	6856
	ATTTCTACAC	CTTTGTAAAT	TATGATAATA	TTTTAATCCC	TAGTTGTTTT	GTTGCTGATC	6916
	CTTAGCCTAA	GTCTTAGACA	CAAGCTTCAG	CTTCCAGTTG	ATGTATGTTA	TTTTTAATGT	6976
	TAATCTAATT	GAATAAAAGT	TATGAGATCA	GCTGTAAAAG	TAATGCTATA	ATTATCTTCA	7036
	AGCCAGGTAT	AAAGTATTTC	TGGCCTCTAC	TTTTTCTCTA	TTATTCTCCA	TTATTATTCT	7096
	CTATTATTT	TCTCTATTTC	CTCCATTATI	GTTAGATAAA	CCACAATTAA	CTATAGCTAC	7156
20	AGACTGAGCC	AGTAAGAGTA	GCCAGGGATG	CTTACAAATT	GGCAATGCTT	CAGAGGAGAA	7216
	TTCCATGTCA	TGAAGACTCT	TTTTGAGTGG	AGATTTGCCA	ATAAATATCC	GCTTTCATGC	7276
	CCACCCAGTC	CCCACTGAAA	GACAGTTAGO	ATATGACCTT	AGTGAAGGTA	CCAAGGGGCA	7336
	ACTTGGTAGG	GAGAAAAAAG	CCACTCTAAA	ATATAATCCA	AGTAAGAACA	GTGCATATGC	7396
	AACAGATACA	GCCCCCAGAC	AAATCCCTCA	GCTATCTCCC	TCCAACCAGA	GTGCCACCCC	7456
	TTCAGGTGAC	AATTTGGAGT	CCCCATTCTA	GACCTGACAG	GCAGCTTAGT	TATCAAAATA	7516
25	CCATAAGAGG	CCTGGGATGG	AAGGGTAGGC	TGGAAAGGGT	TAAGCATGCT	GTTACTGAAC	7576
	Αποιοιοο	GAAGGGAAGG	AGATGGCCA	GCTCAAGCTA	TGTGGGATAG	AGGAAAACTC	7636
	ACCTCCAGAG	CCAGATTCAG	AAACTGGGAT	AAGTCCGAAC	CTACAGGTGG	ATTCTTGTTG	7696
	ACCCACACTG	GTGAAAATGT	TAAGAAGATO	GAAATAATGC	TTGGCACTTA	GTAGGAACTG	7756
	CCCAAATCCA	TATTTGGGG	AGCCTGAAGT	TTATTCAATT	TTGATGGCCC	TTTTAAATAA	7816
30	AAAGAATGTG	CCTCCCCCTG	GTGGCTCACA	CCTGTAATCC	CAGCACTTTG	GGAGGCCGAG	7876
	CCCCCCCAT	CACCTGAAGT	CAGGAGTTCA	AGACCAGCCT	GACCAACATG	GAGAAACCCC	7936
	ATCTCTACTA	AAAATACAAA	ATTACCTCC	CGTGGTGGCA	TATGCCTGTA	ATCCCAGCTA	7996
	CTCGCGAGGC	TGAGGCAGGA	GAATCTTTTC	AACCCGGGAG	GCAGAGGTTG	CGATGAGCCT	8056
	AGATCGTGCC	ATTGCACTCC	AGCCTGGGCA	ACAAGAGCAA	AACTCGGTCT	CAAAAAAAAA	8116
	AAAAAAAAAA	TGAAATTAAC	CARAGGCATT	AGCTTAATAA	TTTAATACTG	TTTTTAAGTA	8176
35	CCCCCCCCCCC	TGGCTGGAAG	AGATCTGTGT	T AAATGAGGGA	ATCTGACATT	TAAGCTTCAT	8236
	CACCATCATA	CCAAATCTGC	TTCTGGAAG	AACTCAATAA	ATATTAGTTG	GAGGGGGGA	8296
	CAGCATCATA	CCTCCACTAG	GACCAGTTT	AGCCCTTGTC	TTTAATCCCT	TTTCCTGCCA	8356
	CTA ATA ACCA	TCTTAGCAGT	GGTTATAAA	GTGGCCTAGG	TTCTAGATAA	TAAGATACAA	8416
	CAGGCCAGGC	ACAGTGGCTC	ATGCCTATA	TCCCAGCACT	TTGGGAGGGC	AAGGCGAGTG	8476
40	TCTCACTTCA	CATCAGGAGT	TCAAGACCAG	CCTGGCCAGC	ATGGCGATAC	TCTGTCTCTA	8536
40	CTANANANA	TACAAAAATT	AGCCAGGCA'	r GGTGGCATGC	ACCTGTAATC	CCAGCTACTC	8596
	CTCACCCTCA	CCCAGAAGAA	TCGCTTGAA	CCAGGAGGTG	TAGGCTGCAG	TGAGCTGAGA	8656
	TCCCACCACT	CCACTCCAGC	CTGGGCGAC	A GAATGAGACT	TTGTCTCAAA	AAAAGAAAAA	8716
	CATACAACAG	CCTACCCTTA	TGTGCTCAC	TTTCACTGTT	GATTACTAGC	TATAAAGTCC	8776
	TATA A ACTO	TTTGGTCAAG	AACCTTGAC	A ACACTAAGAG	GGATTTGCTT	TGAGAGGTTA	8836
4 5	CTCTCACACT	СТСТТТСАТА	TATATACAT	A TACATGTATA	TATGTATCTA	TATCCAGGCT	8896
	TCCCCACCCT	TCCCTCAGAC	TTTCCAGTG	CACTTGGGAGA	TGTTAGGTCA	ATATCAACTT	8956
	TOCCCAGGGI	CAGATTCAAC	CCCTTCTGA	r gtaaaaaaa	AAAAAAAAA	GAAAGAAATC	9016
	CCALAGALI	TCCACCACTC	AAGTTTCAC	CAGGTGGGGCT	TTCCAAGTTG	GGGGTTCTCC	9076
	A ACCOCATOC	CCATTCCTT	CACATCCAT	r TGCTATGTAC	CTTCCCTATG	ATGGCTGGGA	9136
	CACCACYTC	TCAAAACTAG	GAAAGCTAC	T GCCCAAGGAT	GTCCTTACCT	CTATTCTGAA	9196
50	A TOGICANCA	ACTCTCATTA	AAGAGATTG	C CTGTTCTACC	TATCCACACT	CTCGCTTTCA	9256
	VIGIOCKVIV	- ԱՇԱՐԱՐԱՐԱՐԱՐԱՐԱՐԱՐԱՐԱՐԱՐԱՐԱՐԱՐԱՐԱՐԱՐԱՐԱ	Պարասանար	T TTTTTCTTTT	TTTTTGAAAC	GGAGTCTCGC	9316
	VOIGIUVOII						

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TCTGTCGCCC AGGCTAGAGT GCAGTGGCAC GATCTCAGCT CACTGCAAGC TCTGCCTCCC
                                                                               9376
       GGGTTCACGC CATTCTCCTG CCTCACCCTC CCAAGCAGCT GGGACTACAG GCGCCTGCCA
                                                                               9436
       CCATGCCCAG CTAATTTTTT GTATTTTTAG TAGAGACGGG GTTTCACCGT GTTAGCCAGG
                                                                               9496
       ATGGTCTCGA TCTCCTGAAC TTGTGATCCG CCCGCCTCAG CCTCCCAAAG TGCTGGGATT ACAGGCGTGA GCCATCGCAC CCGGCTCAAC TGTAACTTTC TATACTGGTT CATCTTCCCC TGTAATGTTA CTAGAGCTTT TGAAGTTTTG GCTATGGATT ATTTCTCATT TATACATTAG
                                                                               9556
                                                                               9616
       ATTTCAGATT AGTTCCAAAT TGATGCCCAC AGCTTAGGGT CTCTTCCTAA ATTGTATATT
                                                                               9736
       GTAGACAGCT GCAGAAGTGG GTGCCAATAG GGGAACTAGT TTATACTTTC ATCAACTTAG
                                                                               9796
       GACCCACACT TGTTGATAAA GAACAAAGGT CAAGAGTTAT GACTACTGAT TCCACAACTG
10
                                                                               9856
       ATTGAGAAGT TGGAGATAAC CCCGTGACCT CTGCCATCCA GAGTCTTTCA GGCATCTTTG
                                                                               9916
       AAGGATGAAG AAATGCTATT TTAATTTTGG AGGTTTCTCT ATCAGTGCTT AGGATCATGG
                                                                               9976
       GAATCTGTGC TGCCATGAGG CCAAAATTAA GTCCAAAACA TCTACTGGTT CCAGGATTAA
                                                                              10036
       CATGGAAGAA CCTTAGGTGG TGCCCACATG TTCTGATCCA TCCTGCAAAA TAGACATGCT
       GCACTAACAG GAAAAGTGCA GGCAGCACTA CCAGTTGGAT AACCTGCAAG ATTATAGTTT
       CAAGTAATCT AACCATTTCT CACAAGGCCC TATTCTGTGA CTGAAACATA CAAGAATCTG
15
       CATTTGGCCT TCTAAGGCAG GGCCCAGCCA AGGAGACCAT ATTCAGGACA GAAATTCAAG
                                                                              10276
       ACTACTATGG AACTGGAGTG CTTGGCAGGG AAGACAGAGT CAAGGACTGC CAACTGAGCC
                                                                              10336
       AATACAGCAG GCTTACACAG GAACCCAGGG CCTAGCCCTA CAACAATTAT TGGGTCTATT
                                                                              10396
       CACTGTAAGT TTTAATTTCA GGCTCCACTG AAAGAGTAAG CTAAGATTCC TGGCACTTTC
                                                                              10456
       TGTCTCTCT ACAGTTGGCT CAGAAATGAG AACTGGTCAG GCCAGGCATG GTGGCTTACA
                                                                              10516
       CCTGGAATCC CAGCACTTTG GGAGGCCGAA GTGGGAGGGT CACTTGAGGC CAGGAGTTCA
       GGACCAGCTT AGGCAACAAA GTGAGATACC CCCTGACCCC TTCTCTACAA AAATAAATTT
                                                                              10636
       TAAAAATTAG CCAAATGTGG TGGTGTATAC TTACAGTCCC AGCTACTCAG GAGGCTGAGG
                                                                              10696
       CAGGGGGATT GCTTGAGCCC AGGAATTCAA GGCTGCAGTG AGCTATGATT TCACCACTGC
                                                                              10756
       ACTTCTGGCT GGGCAACAGA GCGAGACCCT GTCTCAAAGC AAAAAGAAAA AGAAACTAGA
                                                                              10816
       ACTAGCCTAA GTTTGTGGGA GGAGGTCATC ATCGTCTTTA GCCGTGAATG GTTATTATAG
       AGGACAGAAA TTGACATTAG CCCAAAAAGC TTGTGGTCTT TGCTGGAACT CTACTTAATC
       TTGAGCAAAT GTGGACACCA CTCAATGGGA GAGGAGAGAA GTAAGCTGTT TGATGTATAG
                                                                              10996
       GGGAAAACTA GAGGCCTGGA ACTGAATATG CATCCCATGA CAGGGAGAAT AGGAGATTCG
                                                                              11056
       GAGTTAAGAA GGAGAGGAGG TCAGTACTGC TGTTCAGAGA TTTTTTTTAT GTAACTCTTG
                                                                              11116
       AGAAGCAAAA CTACTTTTGT TCTGTTTGGT AATATACTTC AAAACAAACT TCATATATTC
                                                                              11176
       AAATTGTTCA TGTCCTGAAA TAATTAGGTA ATGTTTTTTT CTCTATAG GAA ATG AAT
30
                                                                              11233
                                                                Glu Met Asn
                                                                85
       CCT CCT GAT AAC ATC AAG GAT ACA AAA AGT GAC ATC ATA TTC TTT CAG
                                                                              11281
       Pro Pro Asp Asn Ile Lys Asp Thr Lys Ser Asp Ile Ile Phe Phe Glu
                90
                                     95
                                                          100
35
       AGA AGT GTC CCA GGA CAT GAT AAT AAG ATG CAA TTT GAA TCT TCA TCA
                                                                              11329
       Arg Ser Val Pro Gly His Asp Asn Lys Met Gln Phe Glu Ser Ser Ser
                                110
                                                     115
       TAC GAA GGA TAC TTT CTA GCT TGT GAA AAA GAG AGA GAC CTT TTT AAA
                                                                              11377
       Tyr Glu Gly Tyr Phe Leu Ala Cys Glu Lys Glu Arg Asp Leu Phe Lys
                            125
40
                                                 130
       CTC ATT TTG AAA AAA GAG GAT GAA TTG GGG GAT AGA TCT ATA ATG TTC
                                                                              11425
       Leu Ile Leu Lys Lys Glu Asp Glu Leu Gly Asp Arg Ser Ile Met Phe
                        140
                                             145
       ACT GTT CAA AAC GAA GAC TAGCTATTAA AATTTCATGC C
                                                                              11464
       Thr Val Gln Asn Glu Asp
45
                    155
```

(18) INFORMATION FOR SEQ ID NO: 18:

(i)SEQUENCE CHARACTERISTICS:
 (A)LENGTH: 471 base pairs
 (B)TYPE: nucleic acid
 (C)STRANDEDNESS: double

	(D)TOPOLOGY: linear																
		(i i	.)MOI	LECUI	E TY	PE:	CDNA	to	mRNA	A.							
5			VODI	· C T \ 7 8	T 50	MIDCE	- •										
		(V)	ORI)				nouse	2									
							live										
		(i)	c)FEA	TURE	::												
0	•	`	(P	AAN (1E/KE		nat p		ide								
			(E) LOC	CATIO	ON:]	L47 CION	71 METI	100·	c							
		(xi	L)SEÇ	QUENC	CE DE	ESCR	IPTIC	ЭИ: 5	SEQ :	ED NO): 18	3:					
5	AAC	ጥጥጥ	GGC	CGA	СТТ	CAC	TGT	ACA	ACC	GCA	GTA	ATA	CGG	AAT	ATA	AAT	48
															Ile		_
	1				5	C M M	~~~		3.03	10	CCM	CMC	mmc	CAC	15	» m.c	0.6
	GAC	CAA	Ual	Leu	Phe	Val	ASD	Lvs	AGA	Gln	Pro	Val	Phe	Glu	GAT Asp	Met	96
20	-			20					25					30			
	ACT	GAT	ATT	GAT	CAA	AGT	GCC	AGT	GAA	CCC	CAG	ACC	AGA	CTG	ATA	ATA	144
	Thr	Asp		Asp	Gln	Ser	Ala	Ser 40	Glu	Pro	Gin	Thr	Arg 45	Leu	Ile	He	
	ጥልሮ	ATG	35 TAC	AAA	GAC	AGT	GAA		AGA	GGA	CTG	GCT		ACC	CTC	TCT	192
25	Tyr	Met	Tyr	Lys	Asp	Ser	Glu	Val	Arg	Gly	Leu	Ala	Val	Thr	Leu	Ser	
		50				. = 0	55		ama	mac	mem	60			3.000	» mm	240
	GTG	AAG	GAT	AGT	AAA	ATG	TCT	ACC Thr	CTC	Ser	TGT	AAG Lve	AAC	AAG Lvc	ATC Ile	TIE	240
	65	цуз	nap	Jer	цуз	70	001		200	001	75	<u>,</u> 0		-,0		80	
	TCC	TTT	GAG	GAA	ATG	GAT	CCA	CCT	GAA	AAT	ATT	GAT	GAT	ATA	CAA	AGT	288
30	Ser	Phe	Glu	Glu		Asp	Pro	Pro	Glu	Asn 90	Ile	Asp	Asp	Ile	Gln 95	Ser	
	GAT	CTC	АТА	ттС	85 ፕፕፕ	CAG	AAA	CGT	GTT		GGA	CAC	AAC	AAG	ATG	GAG	336
	Asp	Leu	Ile	Phe	Phe	Gln	Lys	Arg	Val	Pro	Gly	His	Asn	Lys	Met	Glu	
	-			100					105					110			204
35	TTT	GAA	TCT	TCA	CTG	TAT	GAA	GGA	CAC	TTT	CTT	GCT	TGC	Gln	AAG Lys	GAA	384
	Pne	Giu	115		Leu	TYL	Giu	120	1113	1116	Deu	7120	125	01	ы	010	
	GAT	GAT	GCT	TTC	AAA	CTC	ATT	CTG	AAA	AAA	AAG	GAT	GAA	AAT	GGG	GAT	432
	Asp			Phe	Lys	Leu		Leu	Lys	Lys	Lys		Glu	Asn	Gly	Asp	
	מממ	130 TCT		ΔТС	ттс	ACT	135 CTC	ACT	AAC	тта	CAT	140 CAA	AGT				471
40		Ser															
	145					150					155						
	(19) IN	FORM	ATIO	N FO	R SE	Q ID	NO:	19:								
15							mont	cm t c	· G.								
45		(1					TERI amin										
							o ac										
			(D)TO	POLO	GY:	line	ar									
50		(i	i)MO	LECU	LE T	YPE:	pep	tide									
		. (. \ === x	CMEN	ጥ ጥህ	DC.	M_+a	rmin	al f	raom	ent						

(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 19:

5	Asn 1	Phe	Gly	Arg	Leu 5	His	Cys	Thr	Thr							
	(20) IN	FORM	ATIO	N FO	R SE	Q ID	NO:	20:							
10			(1	A) L: B) T	ENGT: YPE:	H: l ami	RACT 57 a no a lin	mino cid	TICS aci	: ds						
15			ii) xi) :							Q ID	NO:	20:				
	Tyr	Phe	Gly	Lys	Leu	Glu	Ser	Lys	Leu	Ser	Val	Ile	Arg	Asn	Leu	Asn
20	Asp	Gln	Val	Leu 20	Phe	Ile	Asp	Gln	Gly	10 Asn	Arg	Pro	Leu		15 Glu	Asp
	Met	Thr	Asp 35		Asp	Cys	Arg	Asp 40	25 Asn	Ala	Pro	Arg		30 Ile	Phe	Ile
	Ile	Ser 50	Met	Tyr	Lys	Asp	Ser 55		Pro	Arg	Gly	Met 60	45 Ala	Val	Thr	Ile
25	0.5		Lys			70					75	Cys				RΛ
			Phe		85					90					95	Lys
30			Ile	TOO					105					110	Asn	
			Phe 115					120					125			
		130	Arg				135					140		Asp	Glu	Leu
35	145	Asp	Arg	Ser	Ile	Met 150	Phe	Thr	Val	Gln	Asn 155	Glu	Asp			
	(21)	INI	FORMA	4OITA	FOF	SEÇ	QI Q	NO:	21:							
40		()	(E	A) LE B) TY	NGTH	l: 15 amir	ACTER 57 am 10 ac	ino id	CS: acid	ls						
1 5		t)	li) M	OLEC	ULE	TYPE	: pe	ptid	e							
		(2	ci) S	EQUE	NCE	DESC	RIPT	'ION:	SEQ	ID	NO:	21:				
	Tyr 1	Phe	Gly	Lys	Leu 5	Glu	Ser	Lys	Leu	Ser 10	Val	Ile	Arg	Asn	Leu 15	Asn
50			Val	20					25	Asn				30	Glu	
			Asp 35					40	Asn				45	Ile		
55	Ile	Ser 50	Met	Tyr	Lys	Asp	Ser 55	Gln	Pro	Arg	Gly	Met 60	Ala	Val	Thr	Ile

Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile 70 75 Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys 90 85 Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys 105 100 Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu 120 125 Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu 135 140 ٤ Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp 150

(22) INFORMATION FOR SEQ ID NO: 22:

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- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 157 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn 10 Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp 25 Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile 45 40 Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile 60 55 Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile 75 70 Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys 90 Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys 110 105 Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Ser Glu 125 120 Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu 140 135 Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp 150

- (23) INFORMATION FOR SEQ ID NO: 23:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 157 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: peptide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp Met Thr Asp Ser Asp Ser Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile 40. Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile 10 Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile 75 Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys 90 Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys 15 105 Met Gln Phe Glu Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Ser Glu 120 125 Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu 135 140 Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp 20 150

(24) INFORMATION FOR SEQ ID NO: 24:

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- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 157 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn 10 Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp 35 25 Met Thr Asp Ser Asp Ser Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile 40 Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile 55 40 Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Ser Glu Asn Lys Ile 70 Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys 85 90 Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys 45 105 110 Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Ser Glu 120 Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu 135 140 Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp 50 150

(25) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 157 amino acids

- (B) TYPE: amino acid
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

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- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:
- Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp 25 Met Thr Asp Ser Asp Ser Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile 40 Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Ala Glu Asn Lys Ile 75 Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys 90 85 Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys 105 100 Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu 120 Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu 135 Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp 150 145
- (26) INFORMATION FOR SEQ ID NO: 26:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 157 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: peptide
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:
 - Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn 10 Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp 25 20 Met Thr Asp Ser Asp Ser Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile 45 40 Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile 55 Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Ala Glu Asn Lys Ile 75 70 Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys 90 85 Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys 110 105 100 Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Ser Glu 125 120 Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu

														-		
	Glv	130		Sar	T l o	Mot	135		. 1/-1	C1-		140)			
5	145	vəħ	ALG	Ser	116	150		Int	vai	GIU	Asn 155		Asp	•		
	(27) IN	FORM	ATIO	N FO	R SE	Q ID	NO:	27:							
10		((1	EQUE A) L B) T	ENGT YPE:	H: 1 amí	57 a no a	mino cid		ds		•		·		
		(ii)	MOLE	CULE	TYP	E: p	epti	de							
15		(:	xi)	SEQU	ENCE	DES	CRIP	TION	: SE	Q ID	NO:	27:				
	Asn 1	Phe	Gly	Arg	Leu 5	His	Ala	Thr	Thr	Ala 10	Val	Ile	Arg	Asn		Asn
20	Asp	Gln	Val	Leu 20	Phe	Val	Asp	Lys	Arg 25		Pro	Val	Phe	Glu 30	15 Asp	Met
	Thr	Asp	Ile 35		Gln	Ser	Ala	Ser 40		Pro	Gln	Thr	Arg 45	Leu	Ile	Ile
	Tyr	Met 50	Tyr	Lys	Asp	Ser	Glu 55	Val	Arg	Gly	Leu	Ala 60	Val	Thr	Leu	Ser
25	Val 65	Lys	Asp	Ser	Lys	Met 70	Ser	Thr	Leu	Ser	Cys 75		Asn	Lys	Ile	Ile 80
	Ser	Phe	Glu	Glu	Met 85	Asp	Pro	Pro	Glu	Asn 90	Ile	Asp	Asp	Ile	Gln 95	Ser
30				100					105		Gly			110	Met	
			TT2					120			Leu		125	Gln		
		130					135				Lys	140		Asn	Gly	Asp
35	Lys 145	Ser	Val	Met	Phe	Thr 150	Leu	Thr	Asn	Leu	His 155	Gln	Ser			
	(28) INI	FORMA	OITA	V FOR	R SEC	DI	NO:	28:							
40		t)	(E	EQUEN A) LE B) TY	ENGTI (PE:	4: 19 amir	57 an	mino cid		ls						
45		į)	Lí) N	OLE	CULE	TYPE	: pe	eptic	le							
		()	ki) S	SEQUE	ENCE	DESC	CRIPT	: NOI	SEC	OI O	NO:	28:				
	Asn 1	Phe	Gly	Arg	Leu 5	His	Cys	Thr	Thr	Ala 10	Val	Ile	Arg	Asn	Ile 15	Asn
50	Asp	Gln	Val	Leu 20	Phe	Val	Asp	Lys	Arg 25		Pro	Val	Phe	Glu 30	Asp	Met
	Thr	Asp	Ile 35		Gln	Ser	Ala	Ser 40		Pro	Gln	Thr	Arg 45	Leu	Ile	Ile
55	Tyr	Met 50	Tyr	Lys	Asp	Ser	Glu 55		Arg	Gly	Leu	Ala 60	Val	Thr	Leu	Ser
	Val	Lys	Asp	Ser	Lys	Met	Ser	Thr	Leu	Ser	Cys		Asn	Lys	Ile	Ile

						70					75			_	• •	80
	65 Ser	Phe	Glu	Glu	Met	70 Asp	Pro	Pro	Glu	Asn 90		Asp	Asp	Ile	Gln 95	
<i>5</i>	Asp	Leu	Ile	Phe 100	85 Phe	Gln	Lys	Arg	Val 105		Gly	His	Asn	Lys 110		Glu
	Phe	Glu	Ser 115	Ser	Leu	Tyr	Glu	Gly 120	His	Phe	Leu	Ala	Ser 125		Lys	Glu
10	Asp	Asp 130	Ala	Phe	Lys	Leu	Ile 135		Lys	Lys	Lys	Asp 140		Asn	Gly	Asp
	Lys 145	Ser	Val	Met	Phe	Thr 150	Leu	Thr	Asn	Leu	His 155		Ser			
15																
20																
25																
30																
35																•
40																
40																
45																
50																

SEQUENCE LISTING (1) GENERAL INFORMATION: (i) APPLICANT: 5 NAME: KABUSHIKI KAISHA HAYASHIBARA SEIBUTSU KAGAKU KENKYUJO (ii) TITLE OF INVENTION: OSTEOCLASTGENIC INHIBITORY AGENT (iii) NUMBER OF SEQUENCES: 28 10 (iv) ADDRESS: (A) ADDRESSEE: KABUSHIKI KAISHA HAYASHIBARA SEIBUTSU KAGAKU KENKYUJO (B) STREET: 2-3, 1-CHOME, SHIMOISHII 15 (C) CITY: OKAYAMA (E) COUNTRY: JAPAN (F) POSTAL CODE (ZIP): 700 (v) COMPUTER READABLE FORM: (A) MEDIUM TYPE: Floppy disk 20 (B) COMPUTER: IBM PC compatible (C) OPERATING SYSTEM: PC-DOS/MS-DOS (vii) PRIOR APPLICATION DATA: (A1) APPLICATION NUMBER: JP 55,468/1997 25 (B1) FILING DATE: 25-FEB-1997 (2) INFORMATION FOR SEQ ID NO: 1: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 6 amino acids 30 (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (v) FRAGMENT TYPE: internal fragment (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1: 35 Asn Asp Gln Val Leu Phe INFORMATION FOR SEQ ID NO: 2: (3) 40 (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 6 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: internal fragment 45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2: Phe Glu Asp Met Thr Asp 1 5 50 (4)INFORMATION FOR SEQ ID NO: 3: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 7 amino acids (B) TYPE: amino acid 55 (D) TOPOLOGY: linear

	(ii) MOLECULE TYPE: peptide
	(v) FRAGMENT TYPE: internal fragment
5	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:
	Phe Lys Leu Ile Leu Lys Lys
10	(5) INFORMATION FOR SEQ ID NO: 4:
15	(i)SEQUENCE CHARACTERISTICS: (A)LENGTH: 5 amino acids (B)TYPE: amino acid (D)TOPOLOGY: linear
7.5	(ii) MOLECULE TYPE: internal fragment
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:
20	Met Tyr Lys Asp Ser
	(6) INFORMATION FOR SEQ ID NO: 5:
25	(i)SEQUENCE CHARACTERISTICS: (A)LENGTH: 5 amino acids (B)TYPE: amino acid (D)TOPOLOGY: linear
	(ii) MOLECULE TYPE: internal fragment
30	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:
	Ser Thr Leu Ser Cys 1 5
35	(7) INFORMATION FOR SEQ ID NO: 6:
40	(i)SEQUENCE CHARACTERISTICS: (A)LENGTH: 157 amino acids (B)TYPE: amino acid (D)TOPOLOGY: linear
	(ii) MOLECULE TYPE: peptide
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:
45	Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn
	1 5 10 15 Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp
	20 25 30 Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile
50	35 40 45 Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile
	50 55 60 Ser Val Lys Cys Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile
	65 70 75 80 Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys
55	85 90 95 Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys

```
100
                                          105
        Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu
                                      120
                                                          125
        Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu
                                 135
                                                      140
        Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp
        145
                             150
        (8)
             INFORMATION FOR SEQ ID NO: 7:
10
              (i) SEQUENCE CHARACTERISTICS:
                   (A) LENGTH: 157 amino acids
                   (B) TYPE: amino acid
                   (D) TOPOLOGY: linear
15
             (ii) MOLECULE TYPE: peptide
             (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:
        Asn Phe Gly Arg Leu His Cys Thr Thr Ala Val Ile Arg Asn Ile Asn
                                              10
20
        Asp Gln Val Leu Phe Val Asp Lys Arg Gln Pro Val Phe Glu Asp Met
                                          25
        Thr Asp Ile Asp Gln Ser Ala Ser Glu Pro Gln Thr Arg Leu Ile Ile
                                                          45
        Tyr Met Tyr Lys Asp Ser Glu Val Arg Gly Leu Ala Val Thr Leu Ser
25
                                 55
        Val Lys Asp Ser Lys Met Ser Thr Leu Ser Cys Lys Asn Lys Ile Ile
                             70
        Ser Phe Glu Glu Met Asp Pro Pro Glu Asn Ile Asp Asp Ile Gln Ser
                         85
                                              90
        Asp Leu Ile Phe Phe Gln Lys Arg Val Pro Gly His Asn Lys Met Glu
30
                                         105
                                                              110
        Phe Glu Ser Ser Leu Tyr Glu Gly His Phe Leu Ala Cys Gln Lys Glu
                                     120
                                                          125
        Asp Asp Ala Phe Lys Leu Ile Leu Lys Lys Lys Asp Glu Asn Gly Asp
            130
                                 135
                                                      140
        Lys Ser Val Met Phe Thr Leu Thr Asn Leu His Gln Ser
35
        145
                             150
        (9) INFORMATION FOR SEQ ID NO: 8:
             (i) SEQUENCE CHARACTERISTICS:
                   (A) LENGTH: 471 base pairs
40
                   (B) TYPE: nucleic acid
                   (C) STRANDEDNESS: double
                   (D) TOPOLOGY: linear
             (ii) MOLECULE TYPE: CDNA
45
             (vi)ORIGINAL SOURCE:
                   (A) ORGANISM: human
                   (G) CELL TYPE: liver
             (ix) FEATURE:
50
                   (A) NAME/KEY: mat peptide
                   (B) LOCATION: 1..471
                   (C) IDENTIFICATION METHOD: E
             (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:
        TAC TTT GGC AAG CTT GAA TCT AAA TTA TCA GTC ATA AGA AAT TTG AAT
```

		Pne	GLY	ьys	Leu 5	Gru	Ser	пуs	nea	10	vai	116	Arg	ASII	15	-ASII	
	CAC	C D D	GTT	CTC		ATT	GAC	CAA	GGA		CGG	CCT	CTA	TTT		GAT	96
5	Asp	Gln	Val	Leu 20	Phe	Ile	Asp	Gln	Gly 25	Asn	Arg	Pro	Leu	Phe	Glu	Asp	
	N TOC	N CYT	CAT	TCT	GAC	тст	AGA	GAT		GCA	CCC	CGG	ACC		ጥጥጥ	АТТ	144
	Met	Thr	Asp 35	Ser	Asp	Cys	Arg	Asp 40	Asn	Ala	Pro	Arg	Thr	Ile	Phe	Ile	
	מידמ	AGT	ATG	TAT	AAA	GAT	AGC		CCT	AGA	GGT	ATG		GTA	ACT	ATC	192
10	Ile	Ser 50	Met	Tyr	Lys	Asp	Ser 55	Gln	Pro	Arg	Gly	Met 60	Ala	Val	Thr	Ile	
	ருமு		AAG	TGT	GAG	ΔΔΔ		TCA	ACT	CTC	TCC		GAG	AAC	AAA	ATT	240
	Ser 65	Val	Lys	Cys	Glu	Lys	Ile	Ser	Thr	Leu	Ser	Cys	Glu	Asn	Lys	Ile 80	
	ልጥጥ	TCC	TTT	AAG	GAA		AAT	CCT	CCT	GAT	AAC	ATC	AAG	GAT	ACA		288
15	Ile	Ser	Phe	Lys	Glu 85	Met	Asn	Pro	Pro	Asp 90	Asn	Ile	Lys	Asp	Thr 95	Lys	
	AGT	GAC	ATC	ATA	TTC	TTT	CAG	AGA	AGT	GTC	CCA	GGA	CAT	GAT	AAT	AAG	336
	Ser	Asp	Ile	Ile	Phe	Phe	Gln	Arg	Ser	Val	Pro	Gly	His	Asp	Asn	Lys	
		-		100					105					110			
20	ATG	CAA	TTT	GAA	TCT	TCA	TCA	TAC	GAA	GGA	TAC	TTT	CTA	GCT	TGT	GAA	384
			115	Glu				120					125				
	AAA	GAG	AGA	GAC	CTT	TTT	AAA	CTC	ATT	TTG	AAA	AAA	GAG	GAT	GAA	TOU	432
	Lys			Asp	Leu	Pne	135	rea	Tie	Leu	Lys	140	GIU	Asp	GIU	Leu	
25	ccc	CAT	30 מכמ	TCT	ΔΤΔ	ATG		ACT	GTT	CAA	AAC		GAC				471
25	Gly 145	Asp	Arg	Ser	Ile	Met 150	Phe	Thr	Val	Gln	Asn 155	Glu	Asp				
	(10) IN	FORM	OITA	n fo	R SE	Q ID	NO:	.9:								
30		/ i) SEO	UENC	E CH	ARAC	TERT.	STIC	S:								
		(1	(A) LE B) TY D) TO	NGTH PE :	: 11 amin	ami o ac	no a id	cids								
		(i		LECU													
35		·		GMEN			-			raqm	ent						
		•	-	QUEN						_		. =					
	Mat			e Gly													
40	1	- IAI	PHE	: Gly	5 5	, neo	GIU	JCI	Дуз	10	JCI						
	(1:	1) II	NFORM	OITA	N FC	R SE	Q ID	NO:	10:								
45		(:	(QUENC (A) LE (B) T) (D) TC	ENGTH (PE :	1: 10 amir) ami 10 ac	no a	CS: acids	•							
		(ii)M	OLECT	JLE 7	TYPE:	pep	tide	<u>.</u>			,					
50		(v) FR	AGMEI	NT T	YPE:	C-te	ermin	nal 1	ragn	ment						
		. (xi)S	EQUE	NCE I	DESC	RIPT	ON:	SEQ	ID I	NO: 1	10:					,
55	Se 1	r Il	e Me	t Ph	e Th	r Va	l Glr	ı Ası	n Glu	ı Ası 10	Ď						

	(12) INFORMATION FOR SEQ ID NO: 11:
5	(i)SEQUENCE CHARACTERISTICS: (A)LENGTH: 13 amino acids (B)TYPE: amino acid (D)TOPOLOGY: linear
	(ii) MOLECULE TYPE: peptide
10	(v) FRAGMENT TYPE: N-terminal fragment
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:
15	Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg 1 5 10
	(13) INFORMATION FOR SEQ ID NO: 12:
20	(i)SEQUENCE CHARACTERISTICS: (A)LENGTH: 14 amino acids (B)TYPE: amino acid (D)TOPOLOGY: linear
	(ii) MOLECULE TYPE: peptide
25	(v) FRAGMENT TYPE: internal fragment
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:
	Thr Ile Phe Ile Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg 1 5 10
30	(14) INFORMATION FOR SEQ ID NO: 13:
<i>35</i>	(i) SEQUENCE CHARACTERISTICS:(A) LENGTH: 17 amino acids(B) TYPE: amino acid(D) TOPOLOGY: linear
	(ii) MOLECULE TYPE: peptide
	(v) FRAGMENT TYPE: internal fragment
40	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:
	Ile Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys 1 10 15
45	(15) INFORMATION FOR SEQ ID NO: 14:
	(i)SEQUENCE CHARACTERISTICS: (A)LENGTH: 471 base pairs (B)TYPE: nucleic acid
50	(C)STRANDEDNESS: double (D)TOPOLOGY: linear
	(ii) MOLECULE TYPE: CDNA
55	(ix)FEATURE: (A)NAME/KEY: mat peptide (B)LOCATION: 1471 (C)IDENTIFICATION METHOD: S

	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:																		
5	TAC TI Tyr Ph	-														48			
	GAC CA								AAT					GAA		96			
10	ATG AG		TCT										ATA			144			
	50	er Met O	Tyr	Lys	Asp	Ser 55	Gln	Pro	Arg	Gly	Met 60	Ala	Val	Thr	Ile	192			
15	Ser Va	rg AAG al Lys	Ser	Glu	Lys 70	Ile	Ser	Thr	Leu	Ser 75	Ala	Glu	Asn	Lys	Ile 80	240			
	Ile Se	CC TTT er Phe	Lys	Glu 85	Met	Asn	Pro	Pro	Asp 90	Asn	Ile	Lys	Asp	Thr 95	Lys	288			
20	Ser A	AC ATO	Ile 100	Phe	Phe	Gln	Arg	Ser 105	Val	Pro	Gly	His	Asp 110	Asn	Lys	336			
	Met G	AA TTT ln Phe	Glu	Ser	Ser	Ser	Tyr 120	Glu	Gly	Tyr	Phe	Leu 125	Ala	Cys	Glu	384			
25	Lys G 1	AG AGA lu Arg 30 AT AGA	Asp	Leu	Phe	Lys 135	Leu	Ile	Leu	Lys	Lys 140	Glu				432 471			
30		sp Arg														4/1			
	(15)	INFORM	OITA	N FO	R SE	Q ID	NO:	15:											
35		(UENC (A) LE (B) TY (D) TO	NGTH PE :	: 10 amin	ami: o ac	no a id												
		(ii) MC	LECU	LE T	YPE:	pep	tide												
40		(v) FRA							_		c .								
	Tyr P	he Gly	_								J.								
45	_	NFORM	NOITA	FOR	SEQ	ID	NO:	16:											
			(A) LE	NGTH	: 47	1 ba	se p	airs											
50			(B) TY (C) SI (D) TC	RAND	EDNE	SS:	doub												
		(ii)M	OLECU	TLE T	YPE:	cDN	A												
55		(ix)F	EATUR (A) NA (B) LO	ME/K				ide											

(C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

	(XI) SEQUENCE DESCRIPTION: SEQ ID NO: 16:																
5	TAC	TTT	GGC	AAG	CTT	GAA	TCT	AAA	TTA	TCA	GTC	ATA	AGA	AAT	TTG	AAT	48
•	—				5					10					10	Asn	
	GAC	CAA	GTT	CTC	TTC	ATT	GAC	CAA	GGA	AAT	CGG	CCT	CTA	TTT	CAA	GAT	96
10	Asp	GIII	vai	20	Pne .	iie	Asp	GIn	Gly 25	Asn	Arg	Pro	Leu	Phe	Glu	Asp	
	ATG	ACT	GAT	TCT	GAC	TCT	AGA	GAT	AAT	GCA	CCC	CGG	ACC	ATA	TTT	ATT	144
	Met	1111	35	ser	Asp	ser	Arg	Asp	Asn	Ala	Pro	Arg	Thr	Ile	Phe	Ile	
	ATA	AGT	ATG	TAT	AAA	GAT	AGC	CAG	CCT	AGA	GGT	ATG	GCT	GTA	ACT	ATC	192
15	TIE	50	met	ıyr	гуз	Asp	ser 55	GIN	Pro	Arg	Gly	Met	Ala	Val	Thr	Ile	1,72
	TCT	GTG	AAG	TCT	GAG	AAA	ATT	TCA	ACT	CTC	TCC	GCT	GAG	AAC	AAA	ATT	240
	65	vai	гÀг	ser	GIU	Lys 70	IIe	Ser	Thr	Leu	Ser	Ala	Glu	Asn	Lys	Ile	210
	ATT	TCC	TTT	AAG	GAA	ATG	AAT	CCT	CCT	GAT	AAC	ATC	AAG	GAT	ACA		288
20	тте	ser	Pne	гля	61u 85	Met	Asn	Pro	Pro	Asp	Asn	Ile	Lys	Asp	Thr	Lys	200
	AGT	GAC	ATC	ATA	$\mathbf{T}\mathbf{T}\mathbf{C}$	TTT	CAG	AGA	AGT	GTC	CCA	GGA	CAT	GAT	מתמ	DAG	336
	ser	ASD	ire	100	Pne	Pne	GIn	Arg	Ser	Val	Pro	Gly	His	Asp	Asn	Lys	230
	ATG	CAA	TTT	GAA	TCT	TCA	TCA	TAC	GAA	GGA	TAC	TTT	CTA	CCT	тст	GAA	384
25	Mec	GIII	115	GIU	Ser	ser	Ser	120	GLu	Gly	Tyr	Phe	Leu	Ala	Ser	Glu	204
	AAA	GAG	AGA	GAC	CTT	TTT	AAA	CTC	ATT	TTG	AAA	AAA	GAG	GAT	GAA	TTG	432
	пуѕ	130	Arg	Asp	Leu	Pne	Lys 135	Leu	Ile	Leu	Lys	Lys	Glu	Asp	Glu	Leu	132
	GGG	GAT	AGA	TCT	ATA	ATG	TTC	ACT	GTT	CAA	AAC	GAA	GAC				471
30	145	Asp	Arg	Ser	Ile	Met 150	Phe	Thr	Val	Gln	Asn 155	Glu	Asp				
	(18)	INF	DRMAT	NOI	FOR	SEQ	ID N	Ю: 1	.7:								
		/ = 3	0.000	·													
35		(1)		JENCE													
) TYE					paır	S							
			(() STF	ANDE	DNEC	TC 9	loubi									
) TOE					. ~								

- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: genomic DNA
- (vi)ORIGINAL SOURCE:
 - (A) ORGANISM: human
 - (G) CELL TYPE: placenta
- (ix) FEATURE: 45

40

50

- (A) NAME/KEY: 5' UTR
- (B) LOCATION: 1..3
- (C) IDENTIFICATION METHOD: E
- (A) NAME/KEY: leader peptide (B) LOCATION: 4..82
- (C) IDENTIFICATION METHOD: S
- (A) NAME/KEY: intron
- (B) LOCATION: 83..1453
- (C) IDENTIFICATION METHOD: E
- (A) NAME/KEY: leader peptide (B) LOCATION: 1454..1465
- 55 (C) IDENTIFICATION METHOD: S

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(A) NAME/KEY: intron
                     (B) LOCATION: 1466..4848
                     (C) IDENTIFICATION METHOD: E
                     (A) NAME/KEY: leader peptide
                     (B) LOCATION: 4849..4865
                     (C) IDENTIFICATION METHOD: S
                     (A) NAME/KEY: mat peptide
                     (B) LOCATION: 4866..4983
                     (C) IDENTIFICATION METHOD: S
                     (A) NAME/KEY: intron
10
                     (B) LOCATION: 4984..6317
                     (C) IDENTIFICATION METHOD: E
                     (A) NAME/KEY: mat peptide
                     (B) LOCATION: 6318..6451
                     (C) IDENTIFICATION METHOD: S
15
                     (A) NAME/KEY: intron
                     (B) LOCATION: 6452..11224
                     (C) IDENTIFICATION METHOD: E
                     (A) NAME/KEY: mat peptide
                     (B) LOCATION: 11225..11443
                     (C) IDENTIFICATION METHOD: S
20
                     (A) NAME/KEY: 3' UTR
                     (B) LOCATION: 11444..11464
                     (C) IDENTIFICATION METHOD: E
               (xi) SEQUENCE DESCRIPTION: SEO ID NO: 17:
25
         AAG ATG GCT GCT GAA CCA GTA GAA GAC AAT TGC ATC AAC TTT GTG GCA
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              Met Ala Ala Glu Pro Val Glu Asp Asn Cys Ile Asn Phe Val Ala
                                           -30
                                                                   -25
         ATG AAA TTT ATT GAC AAT ACG CTT TAC TTT ATA G
                                                                   GTAAGG CTAATGCCAT
                                                                                            98
         Met Lys Phe Ile Asp Asn Thr Leu Tyr Phe Ile Ala
30
              -20
                                      -15
                                                              -10
         AGAACAAATA CCAGGTTCAG ATAAATCTAT TCAATTAGAA AAGATGTTGT GAGGTGAACT
                                                                                           158
         ATTAAGTGAC TCTTTGTGTC ACCAAATTTC ACTGTAATAT TAATGGCTCT TAAAAAAATA
                                                                                           218
         GTGGACCTCT AGAAATTAAC CACAACATGT CCAAGGTCTC AGCACCTTGT CACACCACGT
                                                                                           278
         GTCCTGGCAC TTTAATCAGC AGTAGCTCAC TCTCCAGTTG GCAGTAAGTG CACATCATGA
                                                                                           338
         AAATCCCAGT TTTCATGGGA AAATCCCAGT TTTCATTGGA TTTCCATGGG AAAAATCCCA
                                                                                           398
35
         GTACAAAACT GGGTGCATTC AGGAAATACA ATTTCCCAAA GCAAATTGGC AAATTATGTA
                                                                                           458
         AGAGATTCTC TAAATTTAGA GTTCCGTGAA TTACACCATT TTATGTAAAT ATGTTTGACA
AGTAAAAATT GATTCTTTTT TTTTTTTCT GTTGCCCAGG CTGGAGTGCA GTGGCACAAT
                                                                                           518
                                                                                           578
         CTCTGCTCAC TGCAACCTCC ACCTCCTGGG TTCAAGCAAT TCTCCTGCCT CAGCCTTCTG
                                                                                           638
         AGTAGCTGGG ACTACAGGTG CATCCCGCCA TGCCTGGCTA ATTTTTGGGT ATTTTTACTA
                                                                                           698
         GAGACAGGGT TTTGGCATGT TGTCCAGGCT GGTCTTGGAC TCCTGATCTC AGATGATCCT
                                                                                           758
40
         CCTGGCTCGG GCTCCCAAAG TGCTGGGATT ACAGGCATGA ACCACCACAC ATGGCCTAAA AATTGATTCT TATGATTAAT CTCCTGTGAA CAATTTGGCT TCATTTGAAA GTTTGCCTTC ATTTGAAACC TTCATTTAAA AGCCTGAGCA ACAAAGTGAG ACCCCATCTC TACAAAAAAAC
                                                                                           818
                                                                                           878
                                                                                           938
         TGCAAAATAT CCTGTGGACA CCTCCTACCT TCTGTGGAGG CTGAAGCAGG AGGATCACTT
                                                                                           998
         GAGCCTAGGA ATTTGAGCCT GCAGTGAGCT ATGATCCCAC CCCTACACTC CAGCCTGCAT
                                                                                          1058
         GACAGTAGAC CCTGACACAC ACACACAAAA AAAAACCTTC ATAAAAAATT ATTAGTTGAC
45
                                                                                          1118
         TTTTCTTAGG TGACTTTCCG TTTAAGCAAT AAATTTAAAA GTAAAATCTC TAATTTTAGA
AAATTTATTT TTAGTTACAT ATTGAAATTT TTAAACCCTA GGTTTAAGTT TTATGTCTAA
ATTACCTGAG AACACACTAA GTCTGATAAG CTTCATTTTA TGGGCCTTTT GGATGATTAT
                                                                                          1178
                                                                                          1238
                                                                                          1298
         ATAATATTCT GATGAAAGCC AAGACAGACC CTTAAACCAT AAAAATAGGA GTTCGAGAAA
                                                                                          1358
         GAGGAGTAGC AAAAGTAAAA GCTAGAATGA GATTGAATTC TGAGTCGAAA TACAAAATTT
                                                                                          1418
50
                                                          CT GAA GAT GAT G
         TACATATTCT GTTTCTCTCT TTTTCCCCCT CTTAG
                                                                                          1470
                                                         Ala Glu Asp Asp Glu
                                                         -10
         GTAGAAATGA ATTTATTTT CTTTGCAAAC TAAGTATCTG CTTGAGACAC ATCTATCTCA CCATTGTCAG CTGAGGAAAA AAAAAAATGG TTCTCATGCT ACCAATCTGC CTTCAAAGAA
                                                                                          1530
                                                                                          1590
         ATGTGGACTC AGTAGCACAG CTTTGGAATG AAGATGATCA TAAGAGATAC AAAGAAGAAC
                                                                                          1650
55
         CTCTAGCAAA AGATGCTTCT CTATGCCTTA AAAAATTCTC CAGCTCTTAG AATCTACAAA
                                                                                          1710
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	ATAGACTTTG C	CCTGTTTCAT	TGGTCCTAAG	ATTAGCATGA	AGCCATGGAT	ТСТСТТСТАС	1770
	GGGGAGCGTT C	SCATAGGAAA	AAGGGATTGA	AGCATTAGAA	TTGTCCAAAA	TCAGTAACAC	1830
	CTCCTCTCAG A	AAATGCTTTG	GGAAGAAGCC	TGGAAGGTTC	CGGGTTGGTG	GTGGGGTGGG	1890
	GCAGAAAATT C	CTGGAAGTAG	AGGAGATAGG	AATGGGTGGG	GCAAGAAGAC	CACATTCAGA	1950
5	GGCCAAAAGC I	rgaaagaaac	CATGGCATTT	ATGATGAATT	CAGGGTAATT	CAGAATGGAA	2010
	GTAGAGTAGG A	AGTAGGAGAC	TGGTGAGAGG	AGCTAGAGTG	ATAAACAGGG	TGTAGAGCAA	2010
	GACGTTCTCT C	CACCCCAAGA	TGTGAAATTT	GGACTTTATC	TTGGAGATAA	TAGGGGTTAAT	2130
	TAAGCACAAT A	ATGTATTAGC	TAGGGTAAAG	ATTAGTTTGT	TGTAACAAAG	ACATCCAAAC	
	ATACAGTAGC T	IGAATAAGAT	AGAGAATTTT	TCTCTCAAAG	AAAGTCTAAG	TACCCACCTC	2190 2250
	AGAAGTAGTA T	TGGCTGGAAG	CAACCTGATG	ATATTGGGAC	CCCCAACCTT	CTTCACTCTT	
10	GTACCCATCA T	CCCCTAGTT	GTTGATCTCA	CTCACATAGT	TGAAAATCAT	CITCAGICII	2310
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	ACTCTAATTG C	GAAGTTAAAC	ACATCAATCC	CCCTCATATT	CCATTCACTA	CARTTERANG	2430
	ACATGGCCAC A	ACCAAGTGCA	AGGAAATCTG	GAAAATATAA	TCTTTATTCC	ACCENC CONT	2490
	ATGACTCTTT A	AAAATTCAGA	TATATATAT	מדע מממדדדד מדי מדי מדי מדי מדי מדי מדי מדי	TCATTCTCCC	TTTCCTATA A	2550
15	AGAATTGATG	TGTGGGGTG	AGGAGGCCAA	AATTAACCCT	TCATICIGGC	TTATTTATA	2610
	TATTACAAGA A	ATGATGGTG	TCATGAATTA	ACCTACACAT	ACCCCACTICA	TIATITIAGT	2670
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	TTAAAGGATG	TACTACAAAC	D A C CTA ATA A	ACAACACACA	GC1GGGGGA	ATACCTACAT	3150
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25	AGCAACAAGG A	CTTTCCTC	TCTCACTCAA	AT CAAGGGCC	AGAACACAGC	TTTTAGATTT	3330
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	CCTAAGCTGC T	rttreneneaer	TACTCATATA	TATCCACATC	ACTONATOR	TIGTACTATA	3750
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1 5	TAGGCAACTT T	FATTCTACCT	ACTTCTCCN N	CACAACATTC	GAGAGTAGGT	TAAAAAACAA	4710
	CTAAAATATA T	TACCATACTT	ACTICIOGAA	TAACAAAGAIIG	TCATTAATAG	TTTTAGAAAA	4770
	ATTTAATGTT T	TACCATACTI TACTCTAC A	A AAC CTG C	אאטאאאטאאא איי	ACIAIGIAIT	TTTAAATGAG	4830
	ATTIMATETT I	Cl	u Nen Ieu (TAN ICA GAT	TAC TIT GGC	AAG CTT	4880
		Gı	.u Ash beu e	era ser wab	Tyr Phe Gly	Lys Leu	
50	GAA TCT AAA	ጥጥል ጥርል ርጥ		አው ምምር አጸጥ	T CAC CAR COM	5	
	Glu Ser Lys	Ten Cer Va	I TIA NOM P	an Iou Aar	AGE CIE	CIC TTC	4928
	ora ser nys	10	T TIE WIG F		Asp Gin Val		
	ልጥጥ ርእር ርእን		ביב ריכיים כייוז יי	15 TT CAA CAT	1 mg 1 cm =:	20	
	ATT GAC CAA	Gly Non N-	or Dra T T	CLI GAA GAT	ATG ACT GAT	TCT GAC	4976
	Ile Asp Gln	GIY ASH AL				Ser Asp	
55	TGT AGA G	25 GTATTTTT		3363776337	35		
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	Cys Arg Asp	
	40	
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	AGTGAATGTG CATTCTTTAA AAATACTGAA TACTTACCTT AACATATATT TTAAATATTT TATTTAGCAT TTAAAAGTTA AAAACAATCT TTTAGAATTC ATATCTTTAA AATACTCAAA	5632
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	Asp Asn Ala Pro Arg Thr Ile Phe Ile	0313
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25	ATA AGT ATG TAT AAA GAT AGC CAG CCT AGA GGT ATG GCT GTA ACT ATC	6391
	Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile	
	TCT GTG AAG TGT GAG AAA ATT TCA ACT CTC TCC TGT GAG AAC AAA ATT Ser Val Lys Cys Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile	6439
	η η η η η η η η η η η η η η η η η η η	
30	ATT TCC TTT AAG GTAAG ACTGAGCCTT ACTTTGTTTT CAATCATGTT AATATAATCA	
	Ile Ser Phe Lys	6496
	ATATAATTAG AAATATAACA TTATTTCTAA TGTTAATATA AGTAATGTAA TTAGAAAACT	6556
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	AAAGIGAATA CITACTAAAA ATTATCAAAC TCTTTACCTA TTGTGATAAT CATCCTTTTTT	6676
25	CIGAGCCIGI CACAGGGGAA GAGGAGATAC AACACTTGTT TTATGACCTC CATCTCCTCA	6736
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40	AGCCAGGTAT AAAGTATTTC TGGCCTCTAC TTTTTCTCTA TTATTCTCCA TTATTATTCT	7096
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4 5	AACAGATACA GCCCCCAGAC AAATCCCTCA GCTATCTCCC TCCAACCAGA GTGCCACCCC	7396
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	AAAAAAAA TGAAATTAAC CAAAGGCATT AGCTTAATAA TTTAATACTG TTTTTAAGTA	8176

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                                                                                        8776
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                                                                                        8836
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                                                                                        8956
                                                                                        9016
                                                                                        9076
15 .
        GTGGTCAACA TCAAAACTAG GAAAGCTACT GCCCAAGGAT GTCCTTACCT CTATTCTGAA
                                                                                        9196
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                                                                                        9256
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        ATTTCAGATT AGTTCCAAAT TGATGCCCAC AGCTTAGGGT CTCTTCCTAA ATTGTATATT
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        GTAGACAGCT GCAGAAGTGG GTGCCAATAG GGGAACTAGT TTATACTTTC ATCAACTTAG
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        AAGGATGAAG AAATGCTATT TTAATTTTGG AGGTTTCTCT ATCAGTGCTT AGGATCATGG
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         Pro Pro Asp Asn Ile Lys Asp Thr Lys Ser Asp Ile Ile Phe Phe Glu
50
                                          95
        AGA AGT GTC CCA GGA CAT GAT AAT AAG ATG CAA TTT GAA TCT TCA TCA
         Arg Ser Val Pro Gly His Asp Asn Lys Met Gln Phe Glu Ser Ser Ser
                                     110
         TAC GAA GGA TAC TTT CTA GCT TGT GAA AAA GAG AGA GAC CTT TTT AAA
         Tyr Glu Gly Tyr Phe Leu Ala Cys Glu Lys Glu Arg Asp Leu Phe Lys
55
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	CTC ATT TTG AAA AAA GAG GAT GAA TTG GGG GAT AGA TCT ATA ATG-TTC	11425												
•	Leu Ile Leu Lys Lys Glu Asp Glu Leu Gly Asp Arg Ser Ile Met Phe 140 145 150													
5	ACT GTT CAA AAC GAA GAC TAGCTATTAA AATTTCATGC C Thr Val Gln Asn Glu Asp 155	11464												
	(19) INFORMATION FOR SEQ ID NO: 18:													
10	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 471 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: double (D) TOPOLOGY: linear													
15	(ii) MOLECULE TYPE: cDNA to mRNA													
	<pre>(vi)ORIGINAL SOURCE: (A)ORGANISM: mouse (G)CELL TYPE: liver</pre>													
20	(ix)FEATURE: (A)NAME/KEY: mat peptide (B)LOCATION: 1471 (C)IDENTIFICATION METHOD: S													
25	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:													
٠	AAC TTT GGC CGA CTT CAC TGT ACA ACC GCA GTA ATA CGG AAT ATA AAT Asn Phe Gly Arg Leu His Cys Thr Thr Ala Val Ile Arg Asn Ile Asn 1 5 10 15	48												
30	GAC CAA GTT CTC TTC GTT GAC AAA AGA CAG CCT GTG TTC GAG GAT ATG Asp Gln Val Leu Phe Val Asp Lys Arg Gln Pro Val Phe Glu Asp Met 20 25 30	96												
	ACT GAT ATT GAT CAA AGT GCC AGT GAA CCC CAG ACC AGA CTG ATA ATA Thr Asp Ile Asp Gln Ser Ala Ser Glu Pro Gln Thr Arg Leu Ile Ile 35 40 45	144												
35	TAC ATG TAC AAA GAC AGT GAA GTA AGA GGA CTG GCT GTG ACC CTC TCT Tyr Met Tyr Lys Asp Ser Glu Val Arg Gly Leu Ala Val Thr Leu Ser 50 55 60	192												
	GTG AAG GAT AGT AAA ATG TCT ACC CTC TCC TGT AAG AAC AAG ATC ATT Val Lys Asp Ser Lys Met Ser Thr Leu Ser Cys Lys Asn Lys Ile Ile 70 75 80	240												
40	TCC TTT GAG GAA ATG GAT CCA CCT GAA AAT ATT GAT GAT ATA CAA AGT Ser Phe Glu Glu Met Asp Pro Pro Glu Asn Ile Asp Asp Ile Gln Ser 85 90 95	288												
4 5	GAT CTC ATA TTC TTT CAG AAA CGT GTT CCA GGA CAC AAC AAG ATG GAG Asp Leu Ile Phe Phe Gln Lys Arg Val Pro Gly His Asn Lys Met Glu 100 105 110	336												
43	TTT GAA TCT TCA CTG TAT GAA GGA CAC TTT CTT GCT TGC CAA AAG GAA Phe Glu Ser Ser Leu Tyr Glu Gly His Phe Leu Ala Cys Gln Lys Glu 115 120 125	384												
50	GAT GAT GCT TTC AAA CTC ATT CTG AAA AAA AAG GAT GAA AAT GGG GAT Asp Asp Ala Phe Lys Leu Ile Leu Lys Lys Lys Asp Glu Asn Gly Asp 130 135 140	432												
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55	(20) INFORMATION FOR SEQ ID NO: 19: (i) SEQUENCE CHARACTERISTICS:													

(A) LENGTH: 9 amino acids

				3) TYE 3) TOE		_										
5		(ii	OM (ECUL	E TY	PE:	pept	ide								
		(v)	FRAC	MENT	TYE	PE: 1	1-tei	cmina	al fr	agme	ent					
		(xi	L) SEC	QUENC	E DE	ESCRI	PTI	ON: S	EQ 1	D NC): 19) :				
10	Asn 1	Phe	Gly	Arg	Leu 5	His	Cys	Thr	Thr							
	(21)	INE	FORMA	MOITA	FOF	SEC	Q ID	NO:	20:							
15		(i	(<i>I</i>	EQUEN A) LE B) TY D) TO	ENGTI PE :	H: 15 amir	57 ar	mino cid		ls						
20		(i	ii) N	OLEC	CULE	TYPE	E: pe	eptio	ie							
		()	ki) S	EQUE	ENCE	DESC	CRIP	: NOI	SEC	QI Ç	NO:	20:				
	Tyr 1	Phe	Gly	Lys	Leu 5	Glu	Ser	Lys	Leu	Ser 10	Val	Ile	Arg	Asn	Leu 15	Asn
25		Gln	Val	Leu 20	Phe	Ile	Asp	Gln	Gly 25		Arg	Pro	Leu	Phe 30	Glu	Asp
	Met	Thr	Asp 35	Ser	Asp	Cys	Arg	Asp		Ala	Pro	Arg	Thr 45		Phe	Ile
	Ile	Ser 50		Tyr	Lys	Asp	Ser 55	Gln	Pro	Arg	Gly	Met		Val	Thr	Ile
30	Ser 65		Lys	Ser	Glu	Lys 70	Ile	Ser	Thr	Leu	Ser 75	Cys	Glu	Asn	Lys	Ile 80
	Ile	Ser	Phe	Lys	Glu 85	Met	Asn	Pro	Pro	Asp 90	Asn	Ile	Lys	Asp	Thr 95	
25	Ser	Asp	Ile	Ile 100	Phe	Phe	Gln	Arg	Ser 105	Val	Pro	Gly	His	Asp 110	Asn	Lys
35	Met	Gln	Phe 115	Glu	Ser	Ser	Ser	Tyr 120	Glu	Gly	Tyr	Phe	Leu 125	Ala	Cys	Glu
	Lys	Glu 130	Arg	Asp	Leu	Phe	Lys 135	Leu	Ile	Leu	Lys	Lys 140	Glu	Asp	Glu	Leu
40	Gly 145	Asp	Arg	Ser	Ile	Met 150	Phe	Thr	Val	Gln	Asn 155	Glu	Asp			
	/00															
	(22			ATIO												
4 5		(() ()	EQUEI A) LI B) T D) T	ENGTI YPE :	H: 19 ami	57 ai	mino cid		ds						
		(ii)	MOLE	CULE	TYP	E: p	eptio	de							
50		(xi)	SEQU	ENCE	DES	CRIP	TION	: SEC	Q ID	NO:	21:				
	_	Phe	Gly	Lys	_	Glu	Ser	Lys	Leu		Val	Ile	Arg	Asn	Leu	Asn
	1 Asp	Gln	Val	Leu	5 Phe	Ile	Asp	Gln		10 Asn	Arg	Pro	Leu		15 Glu	Asp
55	Met	Thr	Asp	Ser	Asp	Ser	Arg	Asp	25 Asn	Ala	Pro	Arg	Thr	30 Ile	Phe	Ile

40 45 Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile 55 60 Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile 70 75 Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys 85 90 Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys 105 Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu 120 125 Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu 135 Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp

(23) INFORMATION FOR SEQ ID NO: 22:

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- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 157 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn 10 Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp 25 Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile 40 3.5 Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile 55 Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile 75 Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys 85 90 Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys 100 105 110 Met Gln Phe Glu Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Ser Glu 120 Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu 135 140 Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp 150

(24) INFORMATION FOR SEQ ID NO: 23:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 157 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn
1 5 10 15
Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp

	Met	Thr	Aso	20 Ser	Asn	Ser	Ara	Δsn	25 Asn	λΙα	Dwo	λ		30	-	 Ile
			35					40					45			
5		50		Tyr			55					60				
	00			Ser		70					75					RΛ
				Lys	85					90					95	
10				Ile 100					105					110	Asn	
			712	Glu				120					125			
15		T30		Asp			135					140		Asp	Glu	Leu
15	Gly 145	Asp	Arg	Ser	Ile	Met 150	Phe	Thr	Val	Gln	Asn 155	Glu	Asp			
	(25)	INI	FORM	ATION	FOF	SEC	Q ID	NO:	24:							
20			I) I)	EQUEN A) LE B) TY	ENGTI (PE :)POLO	H: 19 amir XGY:	7 an 10 ac line	nino cid car	acio	ls						
		()	Ll) r	OLEC	JULE	TYPE	i: pe	ptic	ie							
25		()	(i) S	SEQUE	ENCE	DESC	RIPT	: NOI	SEC) ID	NO:	24:				
	T			Lys	5					10					15	
30				Leu 20					25					3.0		_
30			35	Ser				40					45			
		50		Tyr			55					60				
35	62			Ser		70					75					80
				Lys	85					90					95	
				Ile 100					105					330		
40			112	Glu				120					125			
		130		Asp			T32					140		Asp	Glu	Leu
	145	Asp	Arg	Ser	116	150	Pne	ınr	vaı	GIn	Asn 155	Glu	Asp			
45	(26)	INI	FORM	MOITA	FOF	SEC	DI	NO:	25:							
		(i	(]	EQUEN A) LE B) TY	ENGTH	I: 15	7 aπ	ino		ls						
50) TO												
		· (i	ii) N	10LEC	CULE	TYPE	: pe	ptid	le							

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn 10 15

Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu-Asp 25 Met Thr Asp Ser Asp Ser Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile 40 45 35 Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile 55 Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Ala Glu Asn Lys Ile 70 75 Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys 90 85 Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys 105 100 Met Gln Phe Glu Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu 120 125 115 Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu 135 140 Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp 145

(27) INFORMATION FOR SEQ ID NO: 26:

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- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 157 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn 10 Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp 25 20 Met Thr Asp Ser Asp Ser Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile 40 45 35 Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile 55 60 Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Ala Glu Asn Lys Ile 70 Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys 90 85 Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys 105 110 100 Met Gln Phe Glu Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Ser Glu 115 120 125 Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu 135 140 Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp 150 155

- (28) INFORMATION FOR SEQ ID NO: 27:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 157 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: peptide
- 5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

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Asn Phe Gly Arg Leu His Ala Thr Thr Ala Val Ile Arg Asn Ile Asn
                                                                 15
       Asp Gln Val Leu Phe Val Asp Lys Arg Gln Pro Val Phe Glu Asp Met
                                         25
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       Thr Asp Ile Asp Gln Ser Ala Ser Glu Pro Gln Thr Arg Leu Ile Ile
                                     40
       Tyr Met Tyr Lys Asp Ser Glu Val Arg Gly Leu Ala Val Thr Leu Ser
                                55
       Val Lys Asp Ser Lys Met Ser Thr Leu Ser Cys Lys Asn Lys Ile Ile
10
                                                 75
       Ser Phe Glu Glu Met Asp Pro Pro Glu Asn Ile Asp Asp Ile Gln Ser
                        85
       Asp Leu Ile Phe Phe Gln Lys Arg Val Pro Gly His Asn Lys Met Glu
                    100
                                         105
       Phe Glu Ser Ser Leu Tyr Glu Gly His Phe Leu Ala Cys Gln Lys Glu
15
                115
                                    120
                                                         125
       Asp Asp Ala Phe Lys Leu Ile Leu Lys Lys Lys Asp Glu Asn Gly Asp
                                135
                                                    140
       Lys Ser Val Met Phe Thr Leu Thr Asn Leu His Gln Ser
       145
                            150
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```

(29) INFORMATION FOR SEQ ID NO: 28:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 157 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

30	1				5					10					10	Asn
		Gln		20					25					30	Asp	
		Asp	35					40					45	Leu		
35		Met 50					55					60	Val			
	00	Lys				70					75	Lys				0.0
40		Phe			85					90					O.E.	Ser
.0		Leu		100					105					110	Met	
		Glu	TTO					120	His				125	Gln		
1 5	Asp	Asp 130	Ala	Phe	Lys	Leu	Ile 135	Leu	Lys	Lys	Lys	Asp 140	Glu	Asn	Gly	Asp
	Lys 145	Ser	Val	Met	Phe	Thr 150	Leu	Thr	Asn	Leu	His 155	Gln	Ser			

Claims

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- 1. An osteoclastgenic inhibitory agent, which comprises an interleukin-18 or its functional equivalent.
- 2. The inhibitory agent of claim 1, wherein said interleukin-18 includes the amino acid sequences of SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3 as partial amino acid sequences.
 - 3. The inhibitory agent of claim 1, wherein said interleukin-18 includes the amino acid sequences of SEQ ID NO: 4

and SEQ ID NO: 5 as partial amino acid sequences.

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- 4. The inhibitory agent of claim 1, wherein said interleukin-18 includes the amino acid sequence of SEQ ID NO: 6.
- 5. The inhibitory agent of claim 1. wherein said interleukin-18 is human origin.
 - 6. The inhibitory agent of claim 1, wherein said interleukin-18 includes the amino acid sequence of SEQ ID NO: 7.
 - 7. The inhibitory agent of claim 1, which is a therapeutic agent for osteoclast-related diseases.
 - 8. The inhibitory agent of claim 1, which contains a protein, buffer, or saccharide as a stabilizer.
 - 9. The inhibitory agent of claim 1, which is in the form of a liquid, paste, or solid.
- 15. The inhibitory agent of claim 1, which contains 0.000002-100 w/w % of said interleukin-18.
 - 11. An inhibitory agent as defined in any preceding claim, for use as a pharmaceutical.
- **12.** Use of an inhibitory agent as defined in any of claims 1-10 for the preparation of a medicament effective for treating and/or preventing osteoclast-related diseases.

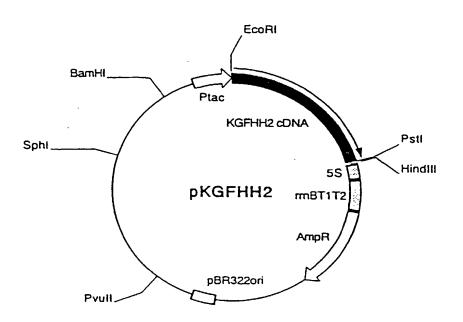


FIG. 1

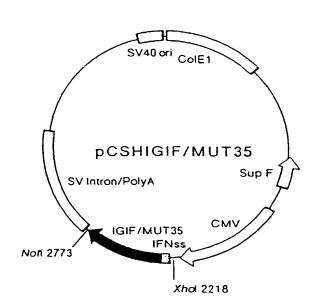


FIG. 2

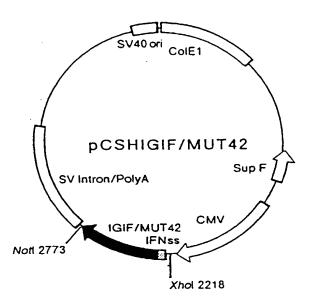


FIG. 3

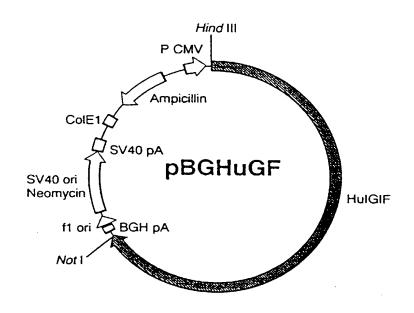


FIG. 4

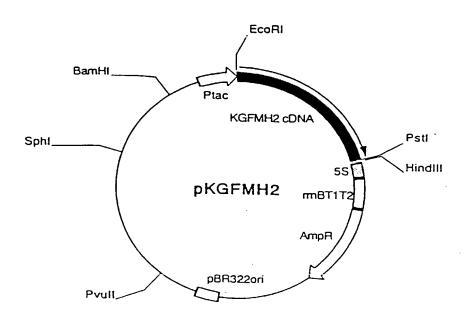


FIG. 5